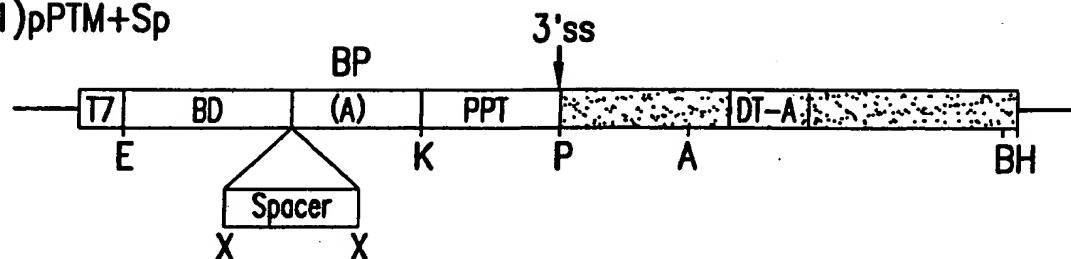


FIG. 1A



(1)pPTM+Sp



(2)pPTM+Sp

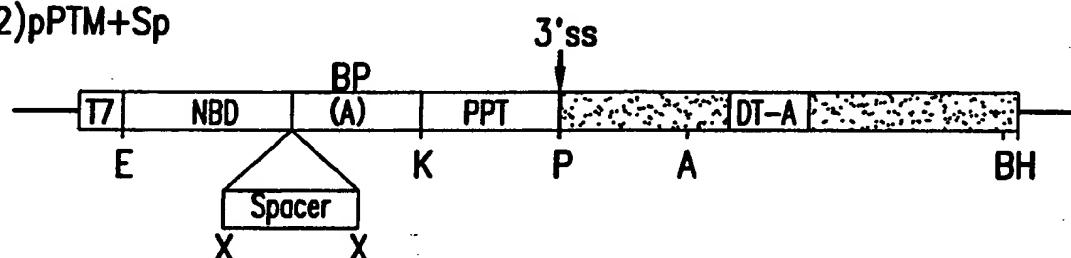
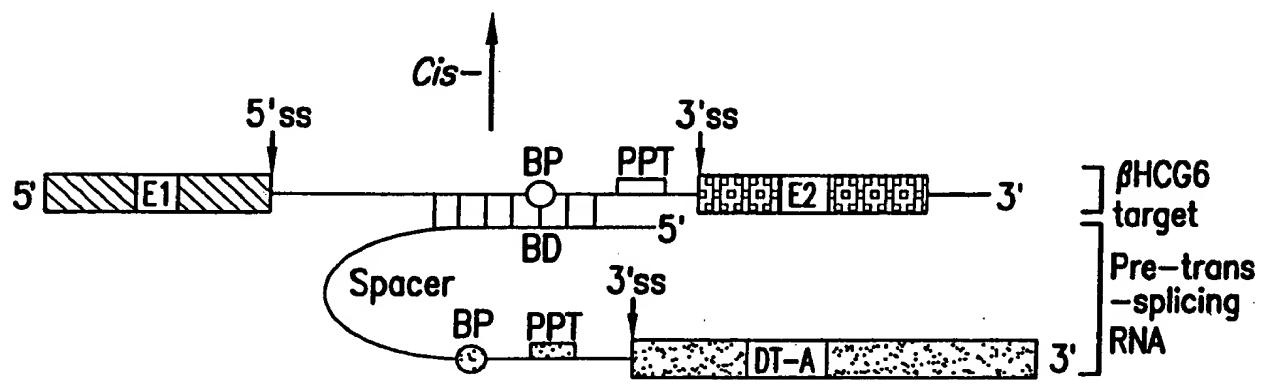


FIG.1B

5' [E1] [ ] [E2] 3'      *Cis*-spliced product



*Trans-*

5' [E1] [ ] [DT-A] 3'      *Trans*-spliced product

FIG.1C

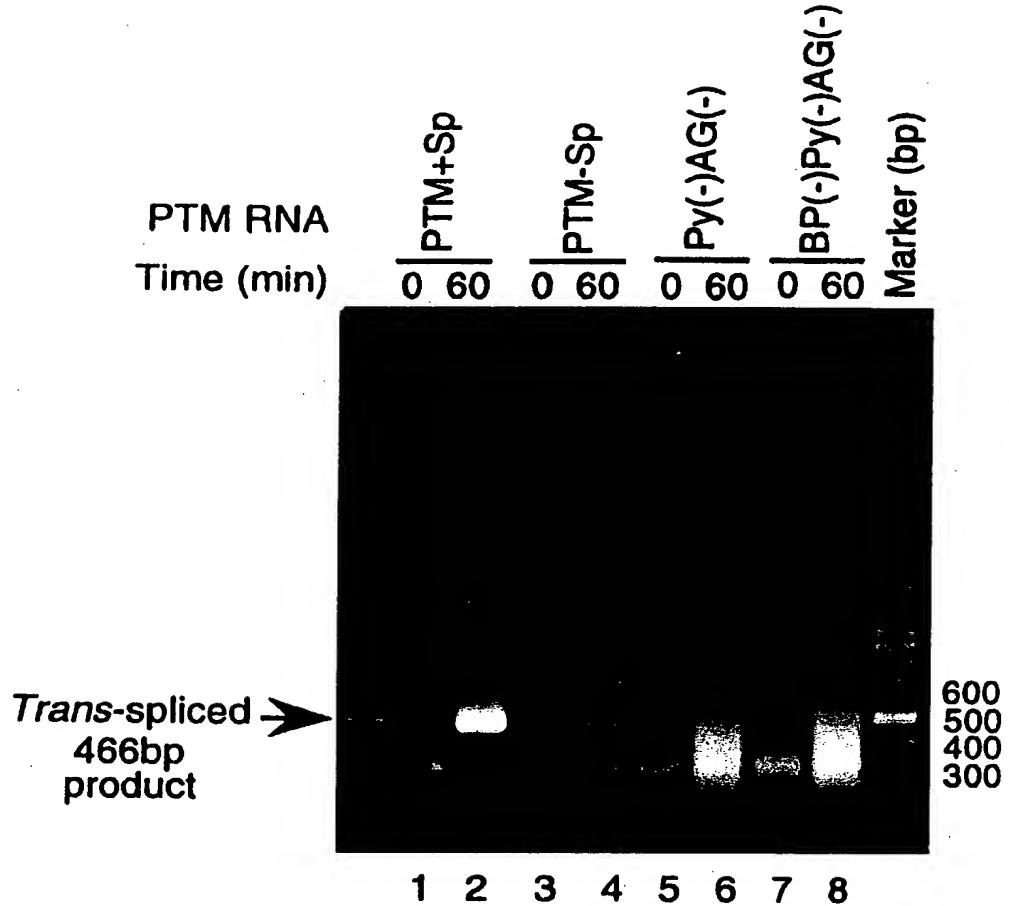


FIG. 2A

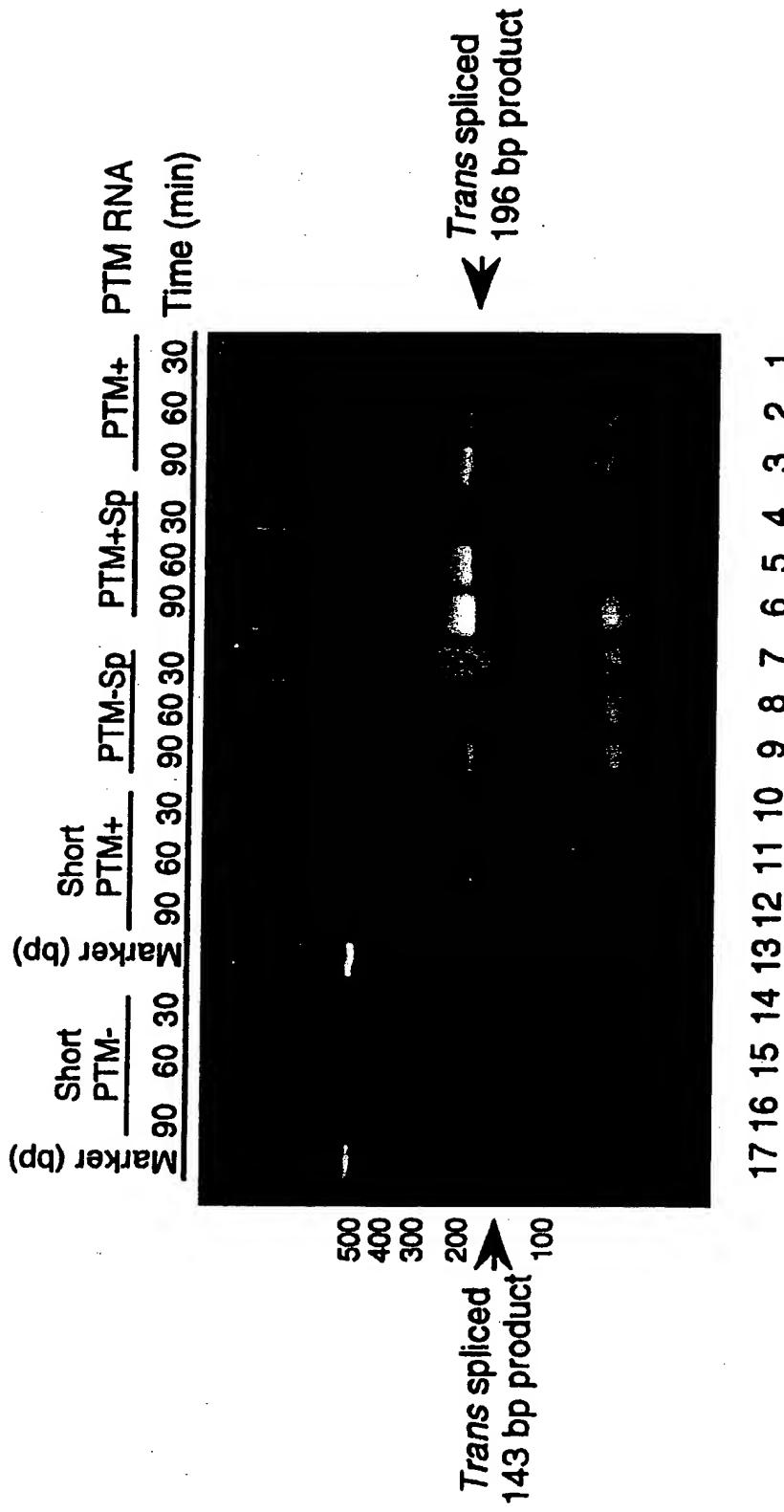


FIG. 2B

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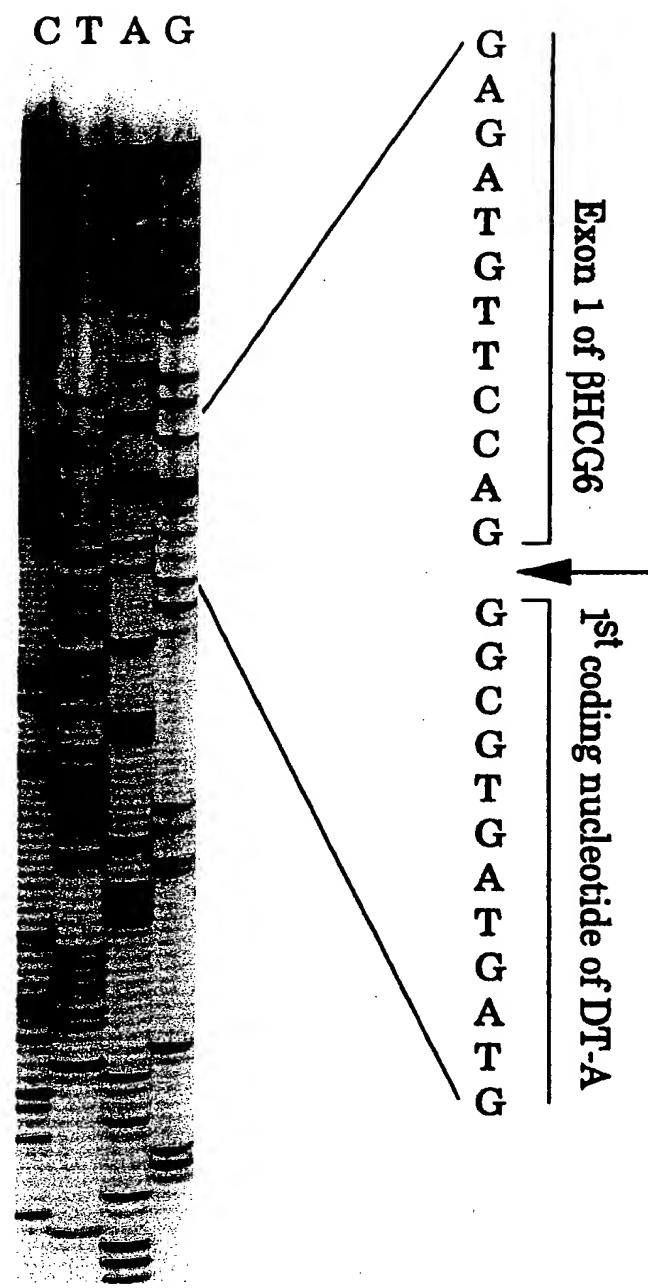
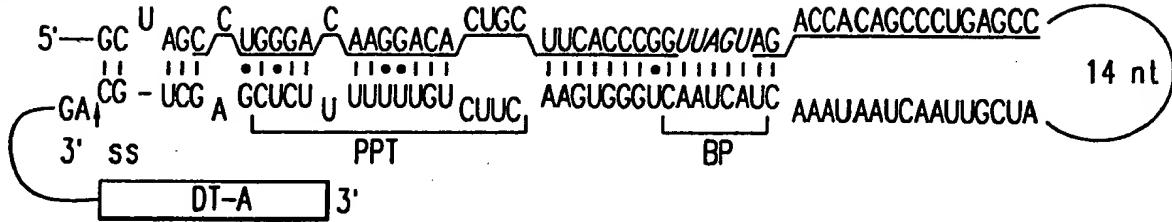


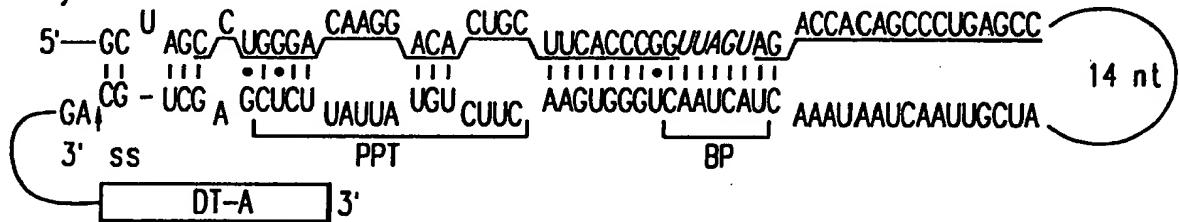
FIG. 3



## 1. PTM+SF :



## 2. PTM+SF-Py1:



### 3. PTM+SF-Py2:

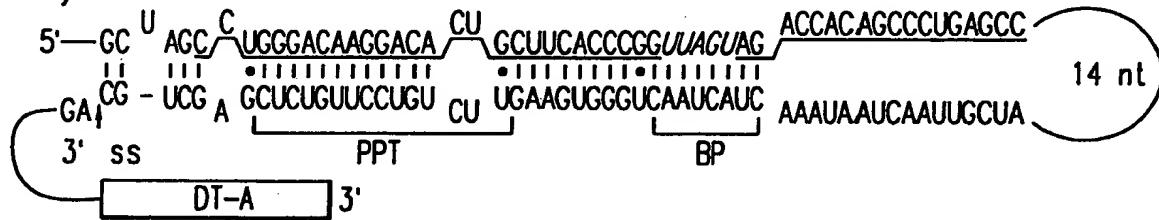


FIG.4A

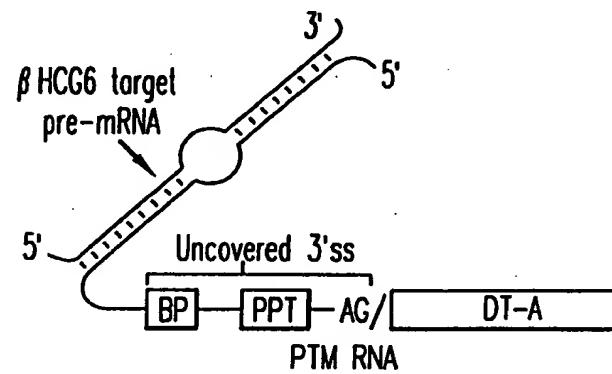


FIG. 4B

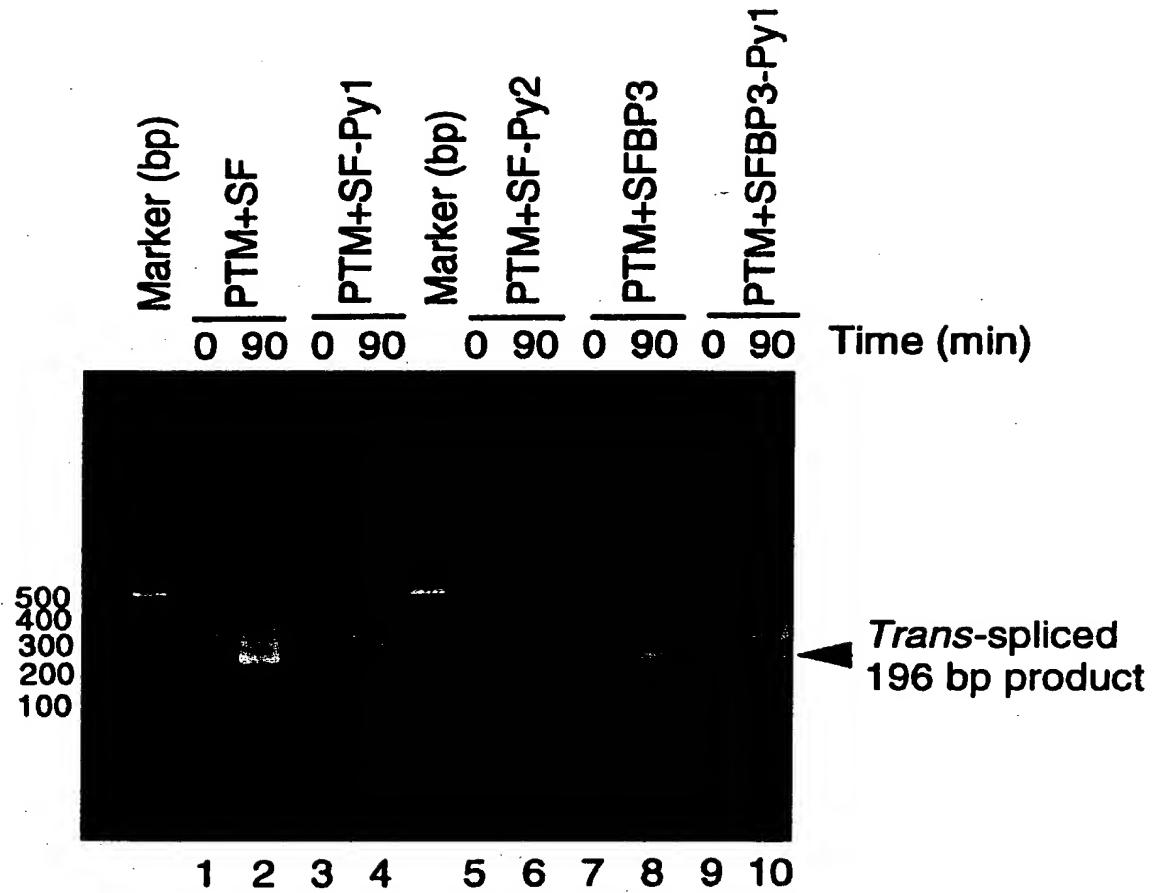


FIG.4C

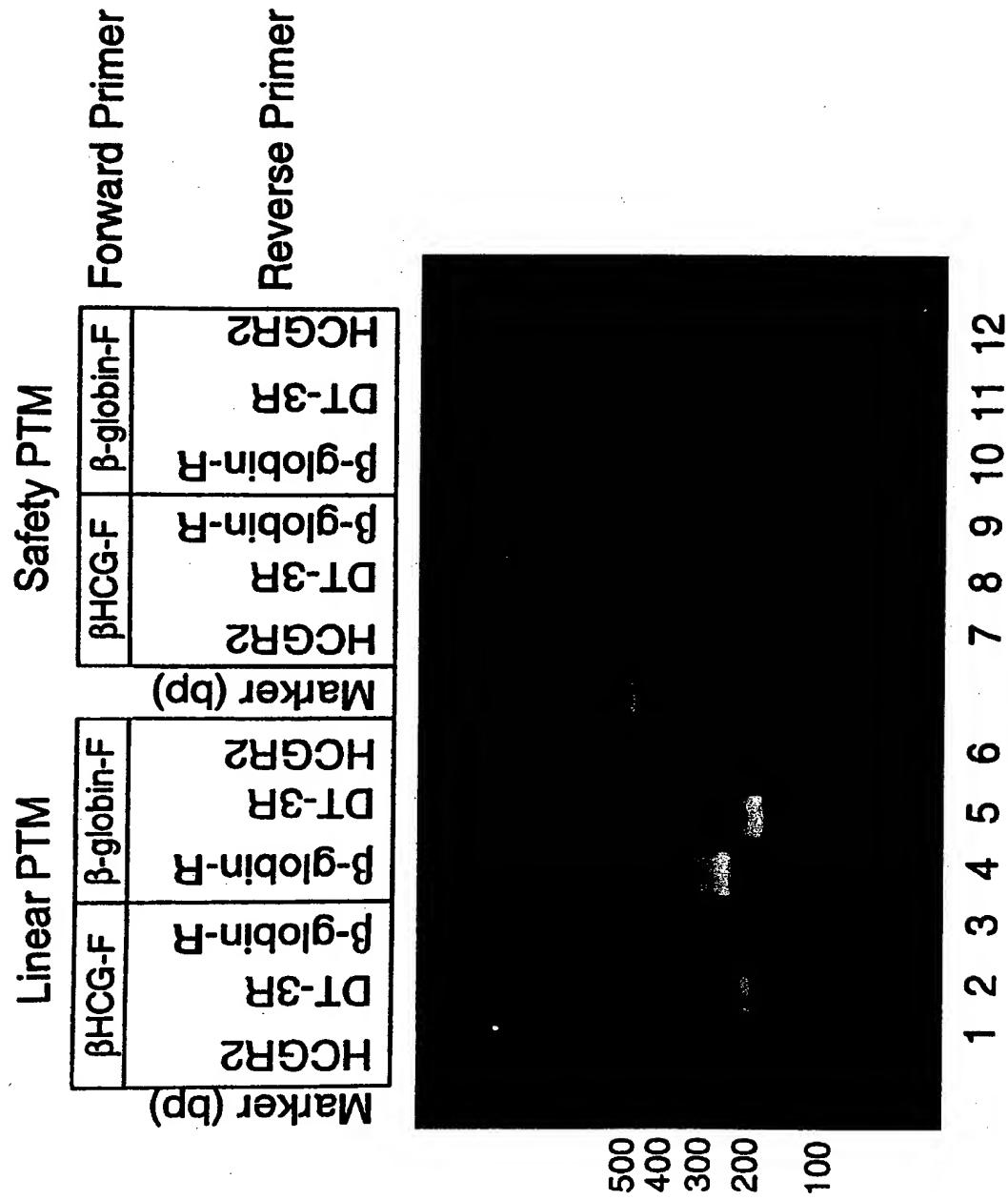
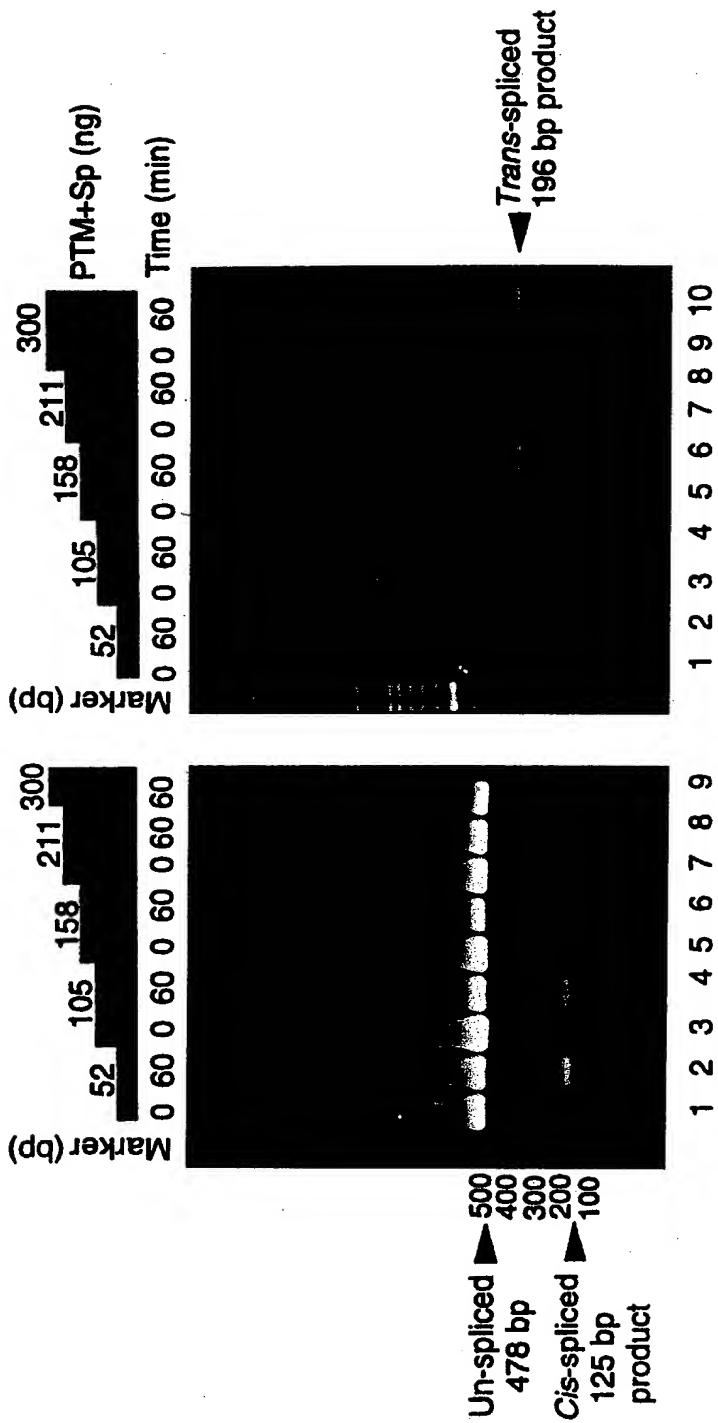
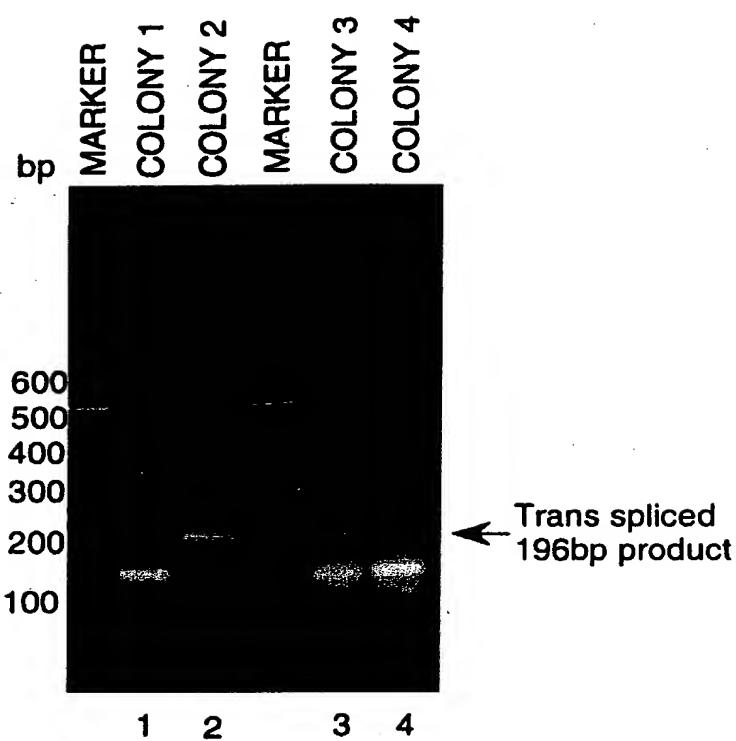


FIG. 5



**FIG. 6A**

**FIG. 6B**

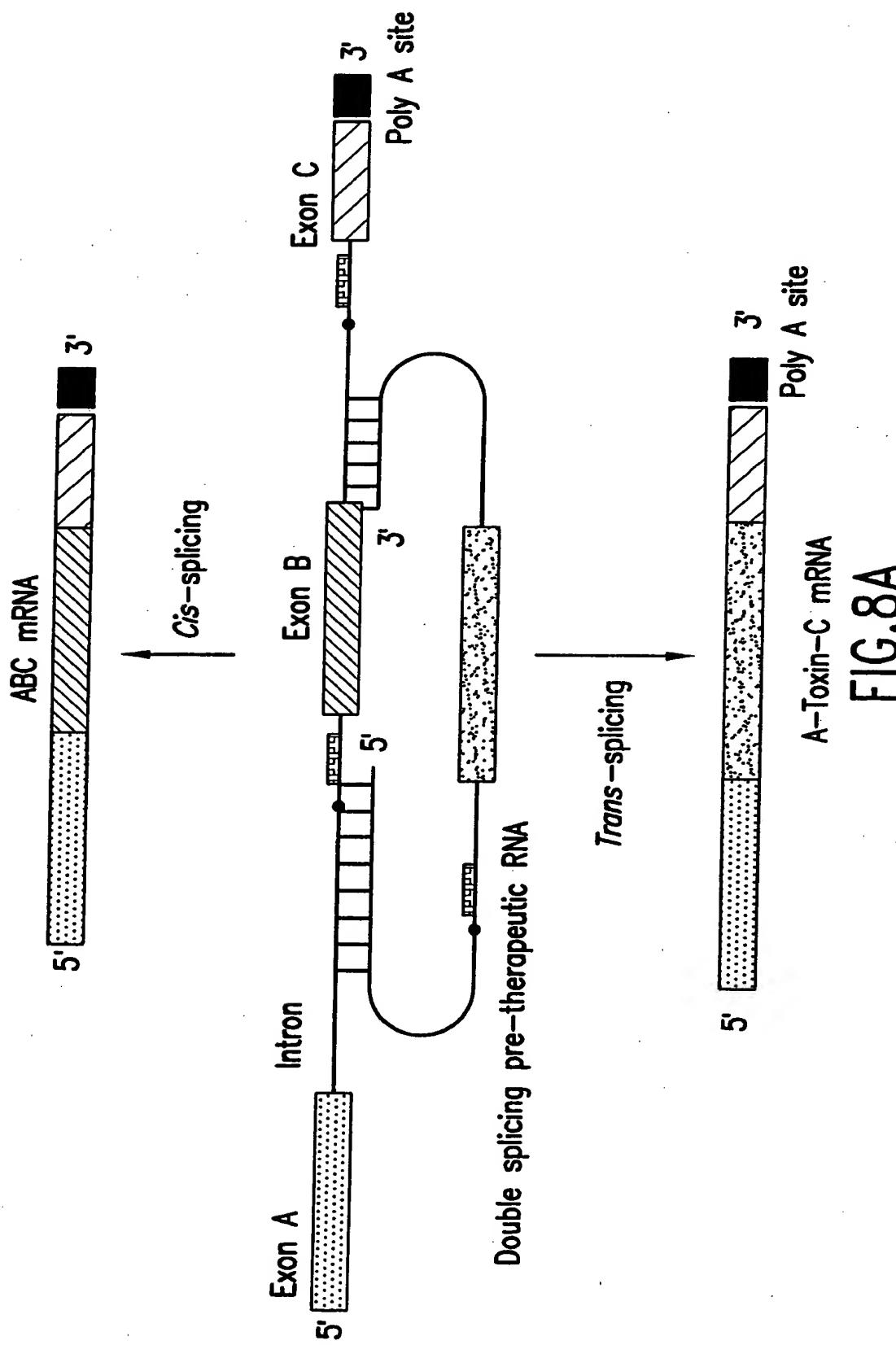


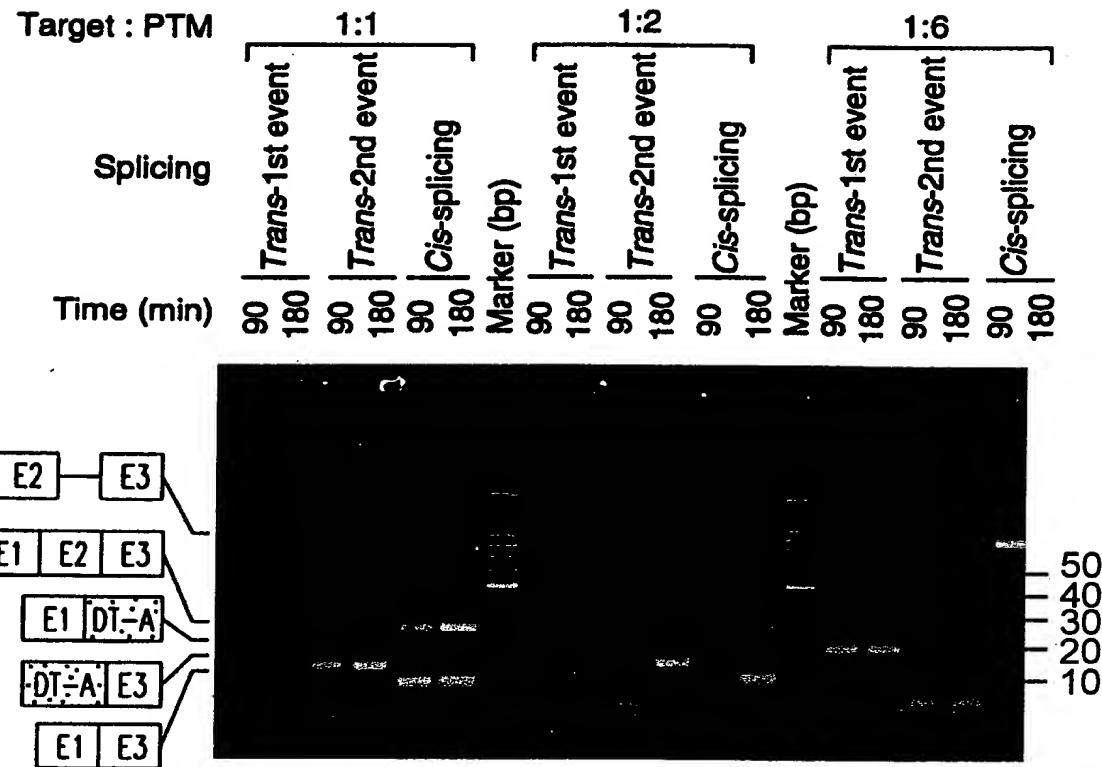
**FIG.7A**



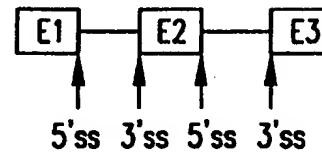
EXON 1 OF  $\beta$ HCG6 |  
5'-CAGGGACGCCAAGGATGGAGATGTCCAG-GGGCTGATGATGTTGTT  
| 1ST CODING NUCLEOTIDE OF DT-A  
GATTCTTAAATCTTTGTGATGGAAAACCTTTCTTGTAACCACGGGACTA  
AACCTGGTTATGTAGATTCCATTCAAAA-3'

FIG. 7B

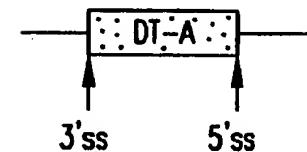




#### $\beta$ HCG TARGET



#### DOUBLE SPlicing PTM



#### Cis-spliced products

**E1 E2 E3** = NORMAL *cis*-SPlicing (277bp)

**E1 E3** = Exon SKIPPING (110bp)

#### Trans-spliced products

**E1 DT-A** = 1st EVENT, 196bp. *Trans*-SPlicing BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

**DT-A E3** = 2nd EVENT, 161bp. *Trans*-SPlicing BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

**FIG.8B**

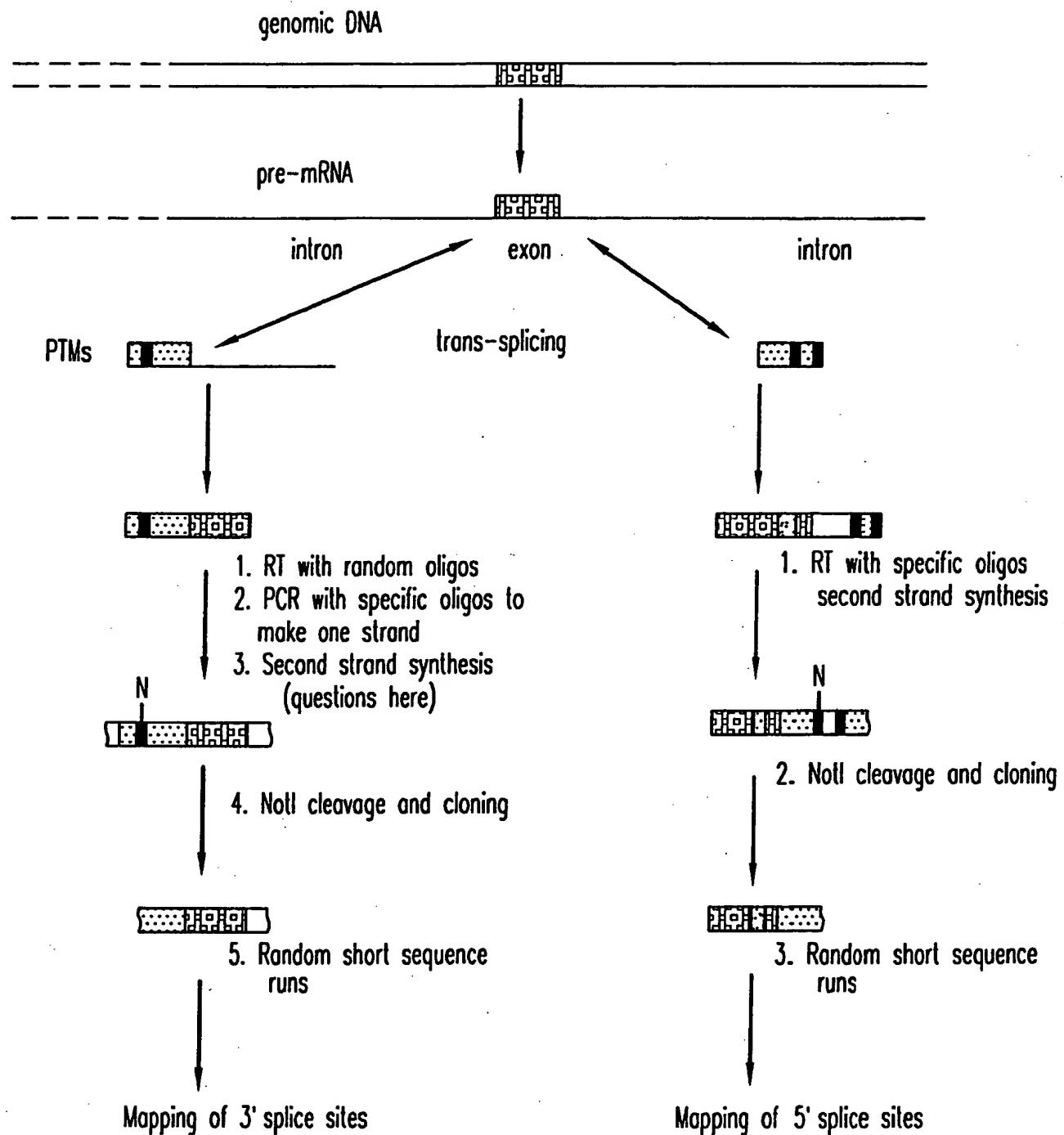
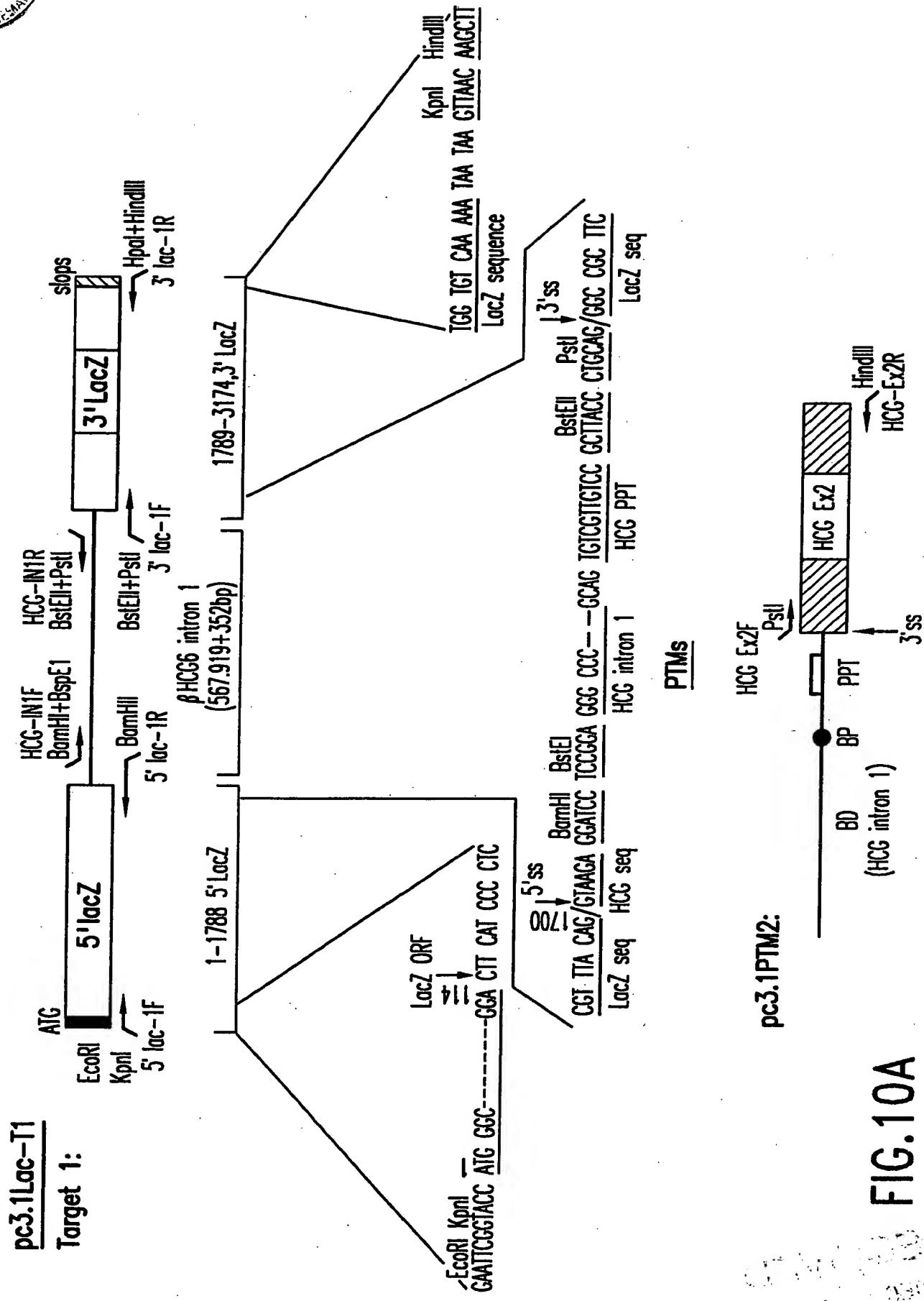


FIG.9



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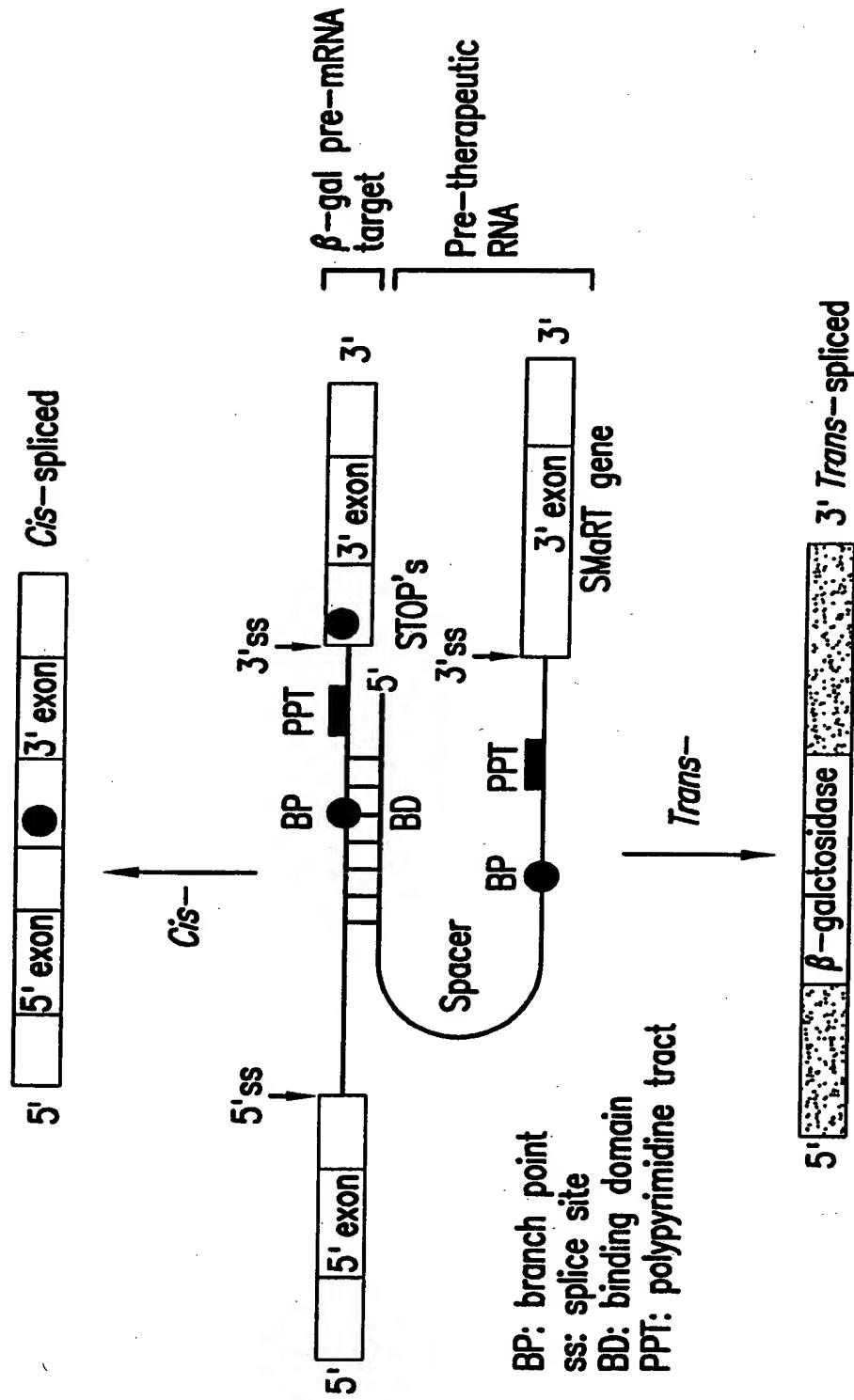


FIG. 10B

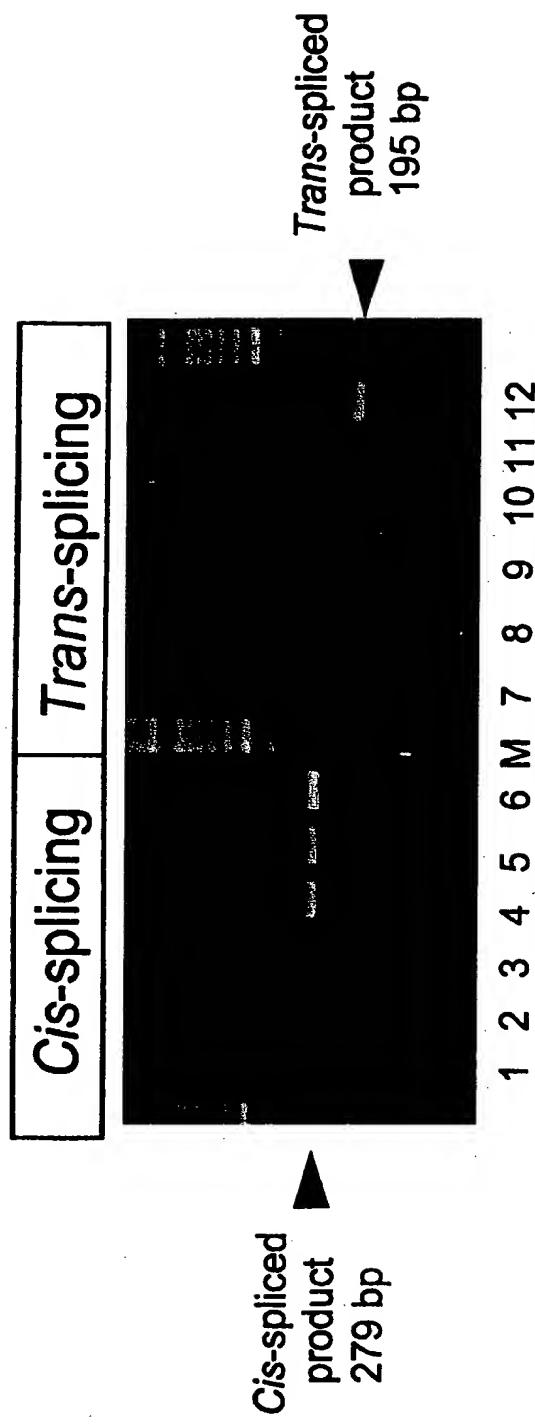
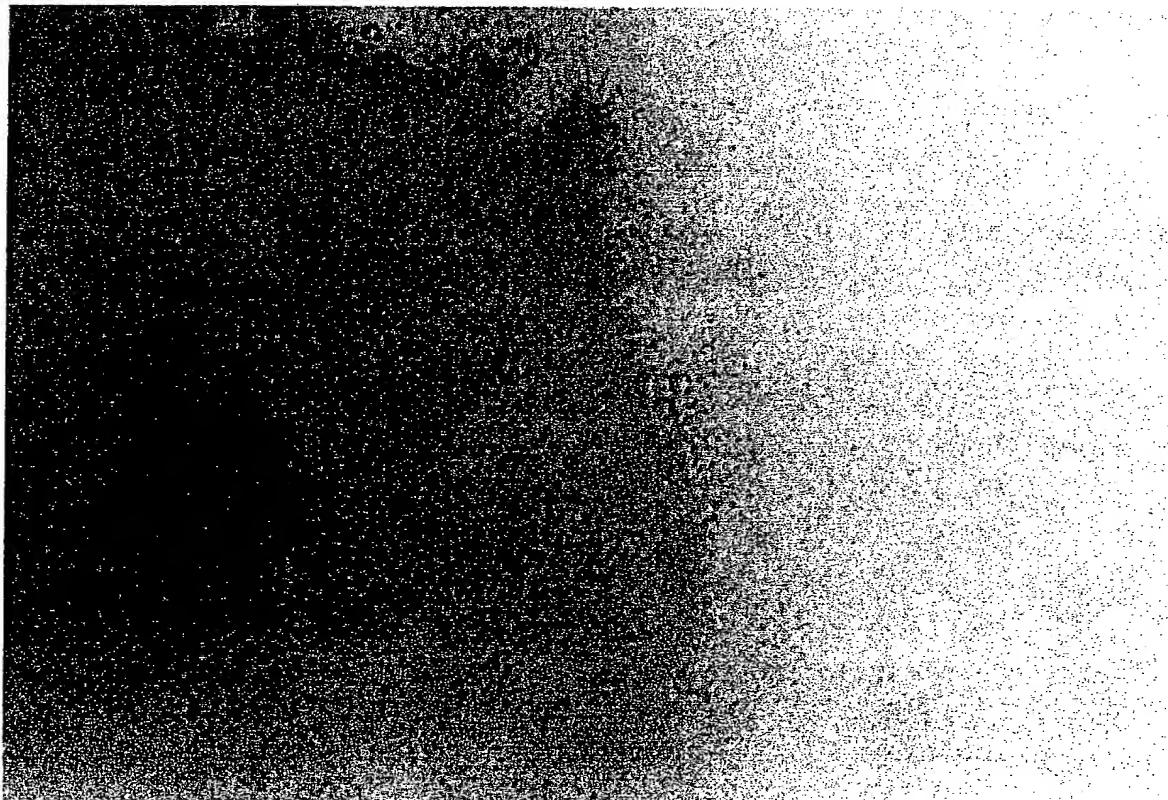
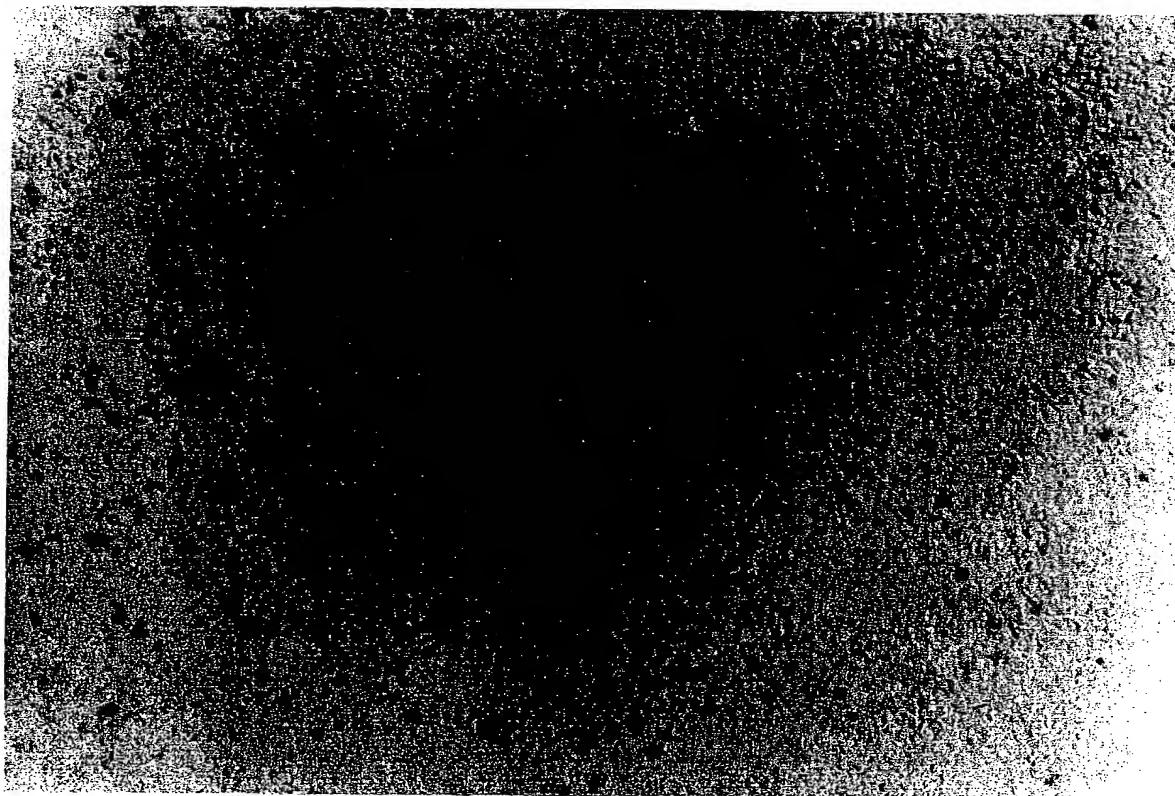


FIG. 11A



**FIG.11B**



**FIG. 11C**

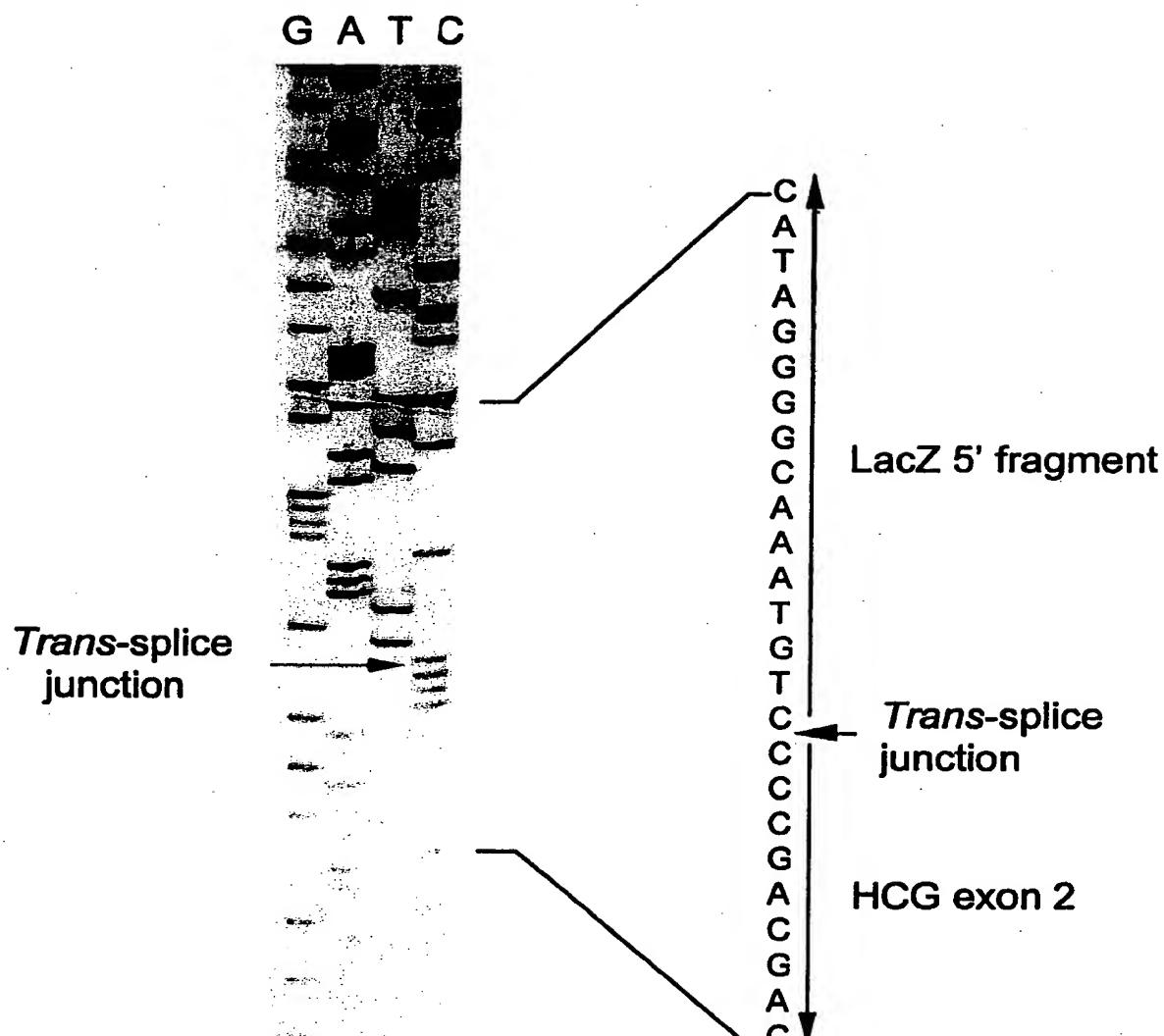


FIG. 12A



1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

BioLac-TR1

GGCTTTCGCTACCTGGAGAGACGGCGCGCTGATCCTTTCGAAATACGCCAACGGATGGCTAACAGTCTTG

Splice junction

GGGTTTCGCTAAACTGGCAGGCTTTCAGTATCCCGTTACAG/GCCGCTTCGCTCAATAATG  
GGACTGGGTGATCAGTGGATTAAATATGATAAAACGGCAACCCGTTACGGCGGTGATT  
TGGCGATACGCCAACGATGCCAGTTCTGATGAACGGTCTGGCTTTGCCGACCCAGGCCATCCAG

Lac-TR2

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

BioLac-TR1

GGCTTTCGCTACCTGGAGAGACGGCGCGCTGATCCTTTCGAAATACGCCAACGGATGGCTAACAGTCTTG

Splice junction

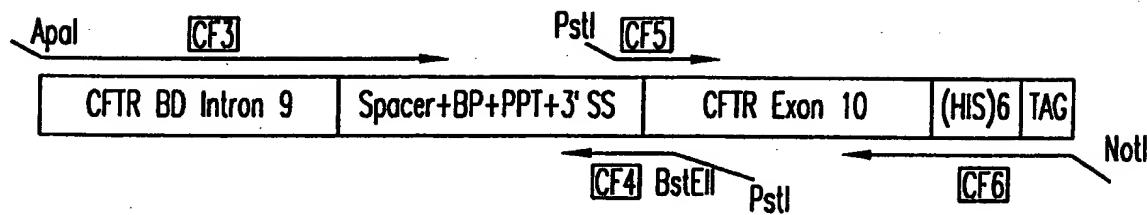
GGCTTTCGCTAAACTGGCAGGCTTTCAGTATCCCGTTACAG/GCCGCTTCGCTGCTGCTGCTGCT  
GAGCATGGCGGACATGGCATCCAAGGACCCACTTOGCCAACGGTGCCT

HCGR2

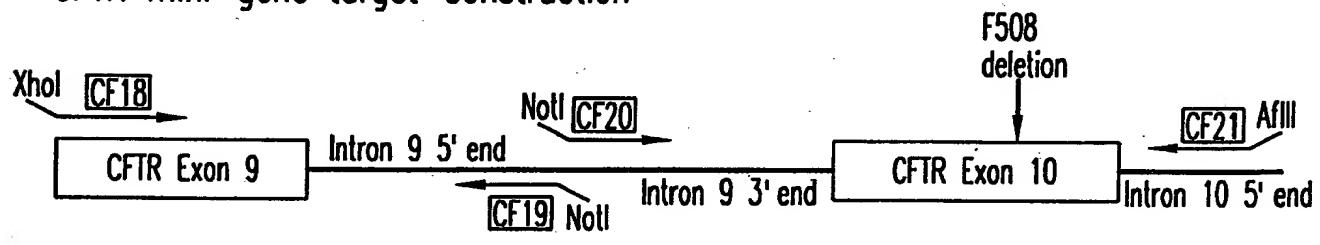
FIG. 12B



### CFTR Pre-therapeutic molecule (PTM or "bullet")

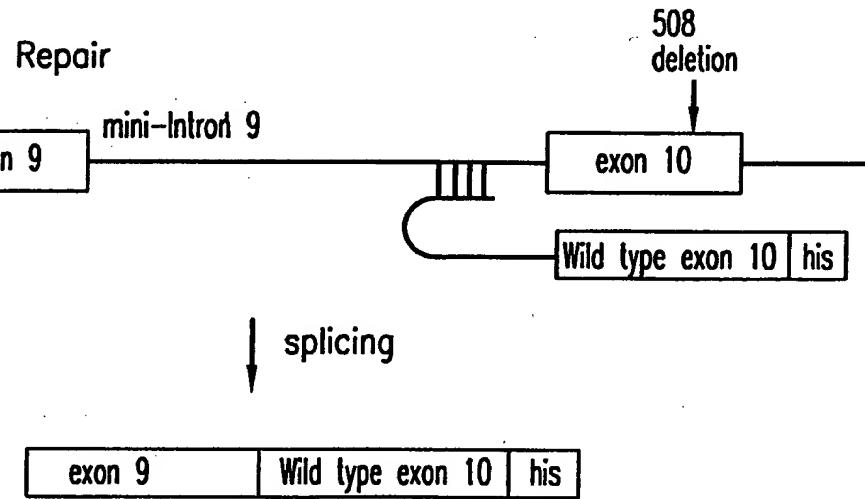


### CFTR mini-gene target-construction



### Trans-splicing Repair

Binding  
of  
PTM to Target



**FIG.13**



primer pairs

→

Lane	Primer Pair
-	18+28
1	18+Biohis 2
2	18+Biohis
3	18+Biohis
4	8+28
5	8+Biohis 2
6	8+Biohis
standard	

**FIG. 14**



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DNA sequence 500 b.p. GCTAGCGTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

FIG. 15A

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TAAACCGCTGATCAGCCCTCGACTGTGCCTCTAGTTGCCCATCTGCCCTCCCCATCTGTTGCCCTCCTTGACC  
 ATTTGGGACTAGTCGGAGCTGACACGGAAAGATCAACCGTCGTAGACAACAAACGGGAGGGGCAOGGAAGGAACTGG  
 410 CF27

CTGGAAAGGTCCCACTCCCCAC 500  
GACCTTCCACGGTGAGGGTG

## Restriction Endonucleases site usage

Acc	I	EcoR	I	Sau96	I	2
Apa	I	EcoR	V	Sca	I	1
Apal	I	Hae	II	Sma	I	-
Avr	II	Hae	III	Sph	I	1
BamH	I	HinC	II	Spi	I	-
Ban	II	HinD	III	Ssp	I	-
Bbe	I	Hinf	I	Stu	I	-
-	-	Nde	I	-	-	-
-	-	Nhe	I	-	-	-
-	-	Not	I	-	-	-
-	-	PfIM	I	-	-	-
-	-	Pst	I	-	-	-
-	-	Pvu	I	-	-	-
-	-	Pvu	II	-	-	-

**FIG. 15B**



PTM [CFTR BD Intron 9] Spacer+BP+PPT+3' SS [CFTR exons 10-24] (His) 6 TAG

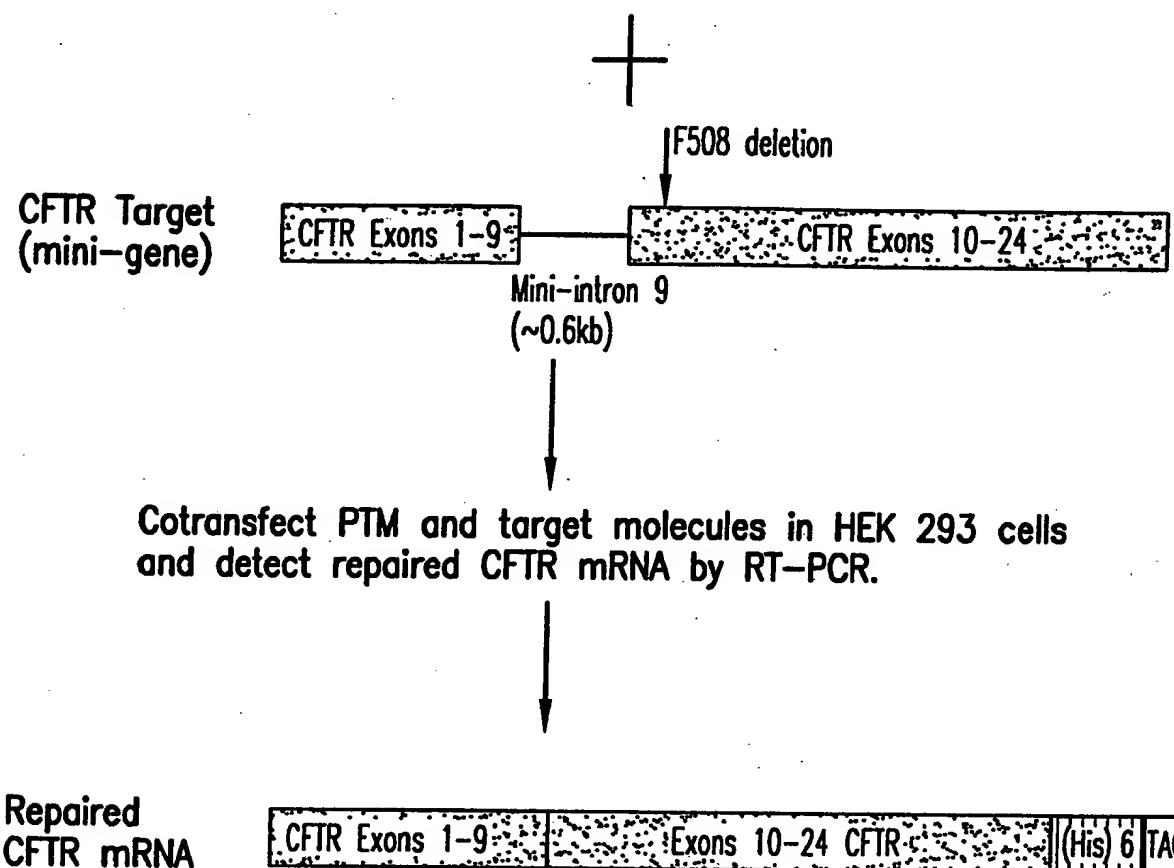


FIG. 16

Double Splicing  
PTM

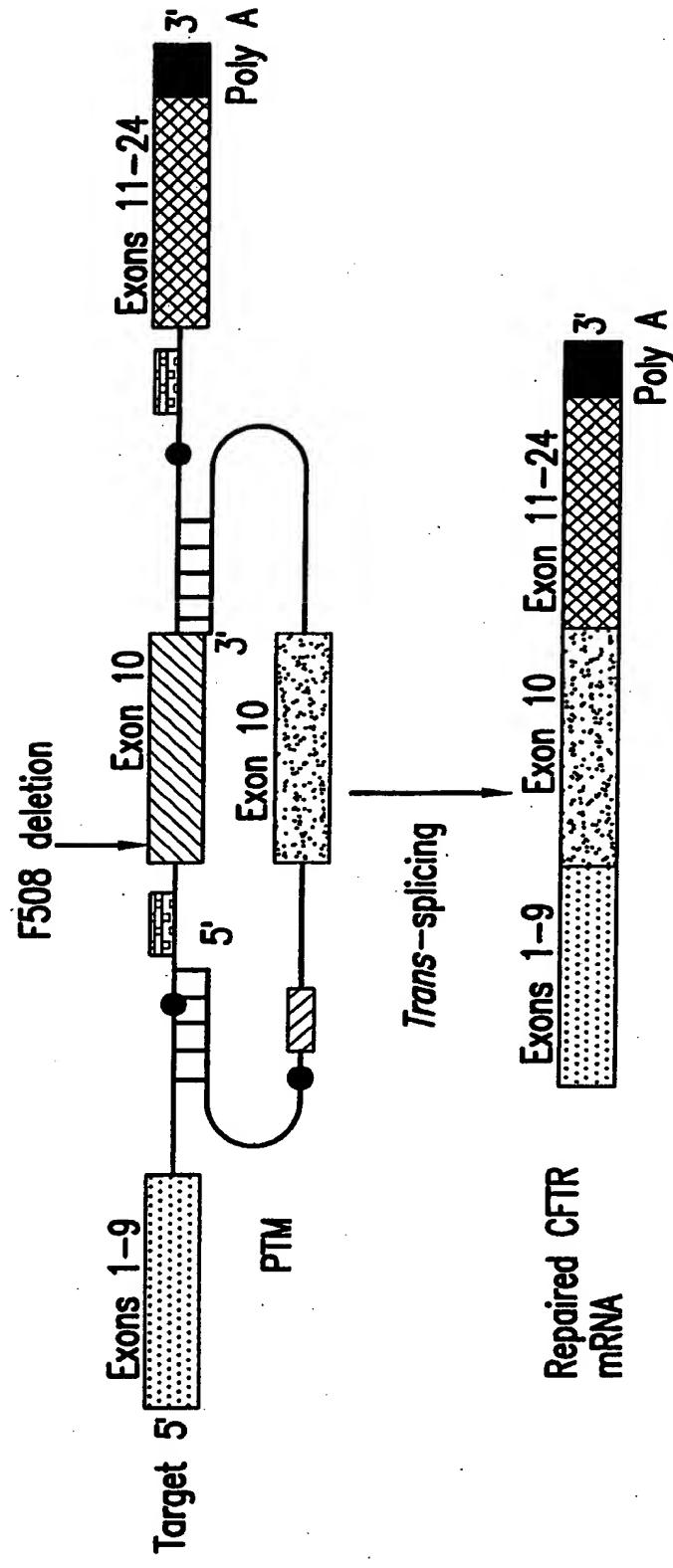
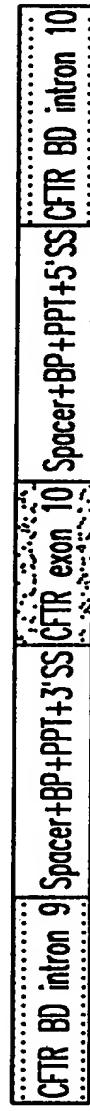


FIG. 17



DOUBLE TRANS-SPLICING SPECIFIC TARGET

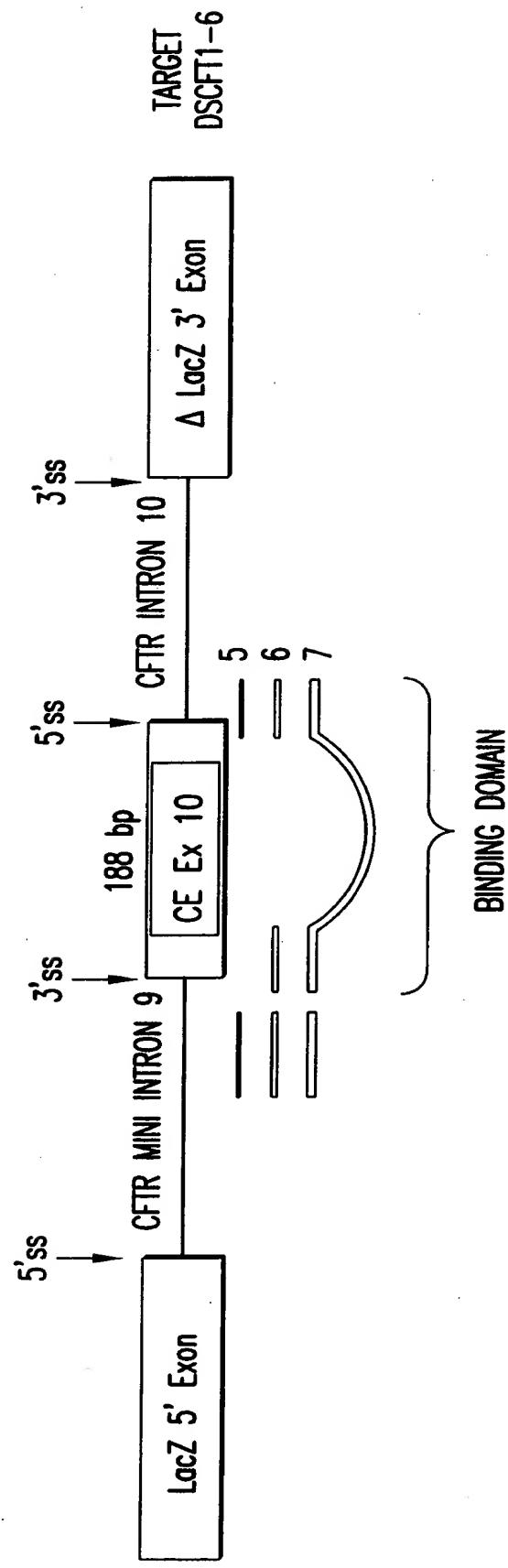


FIG. 18



## DOUBLE TRANS-SPlicing PTMs

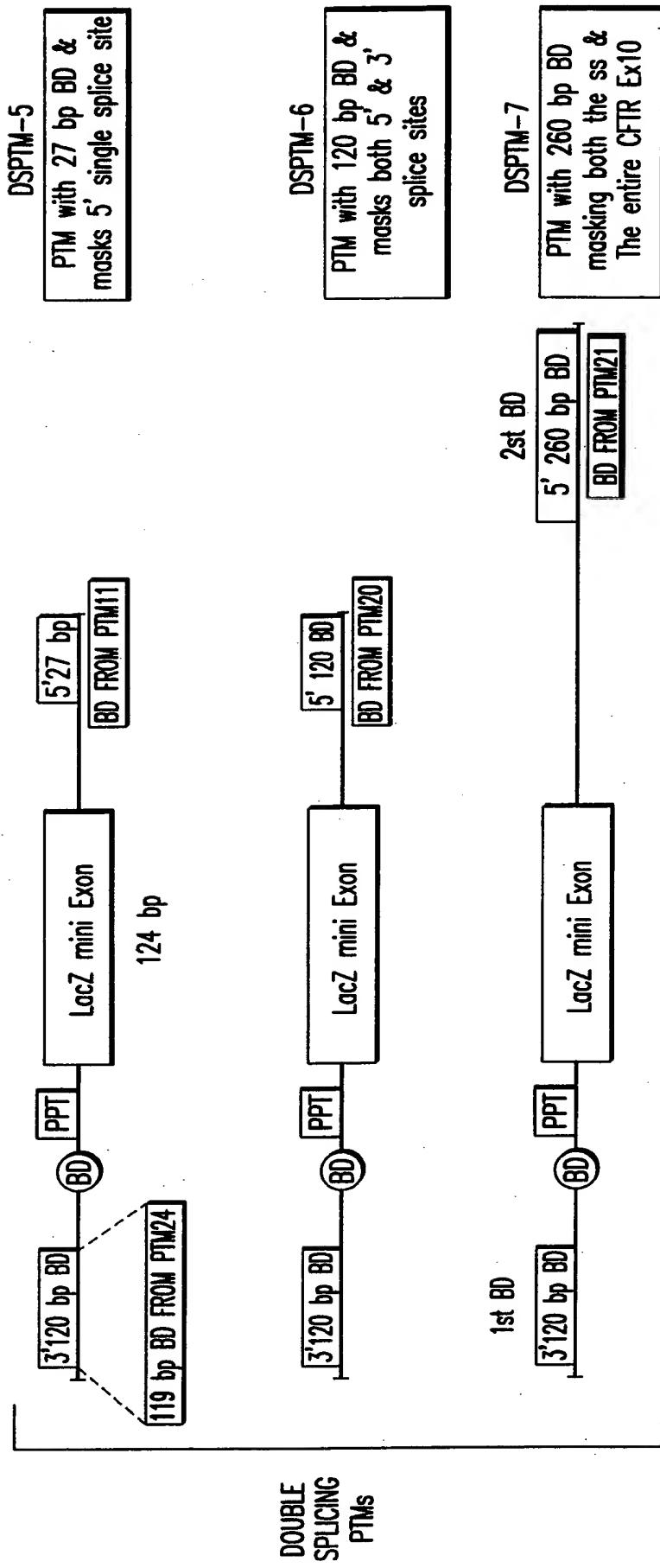


FIG. 19



### DOUBLE TRANS-SPlicing $\beta$ -GAL MODEL

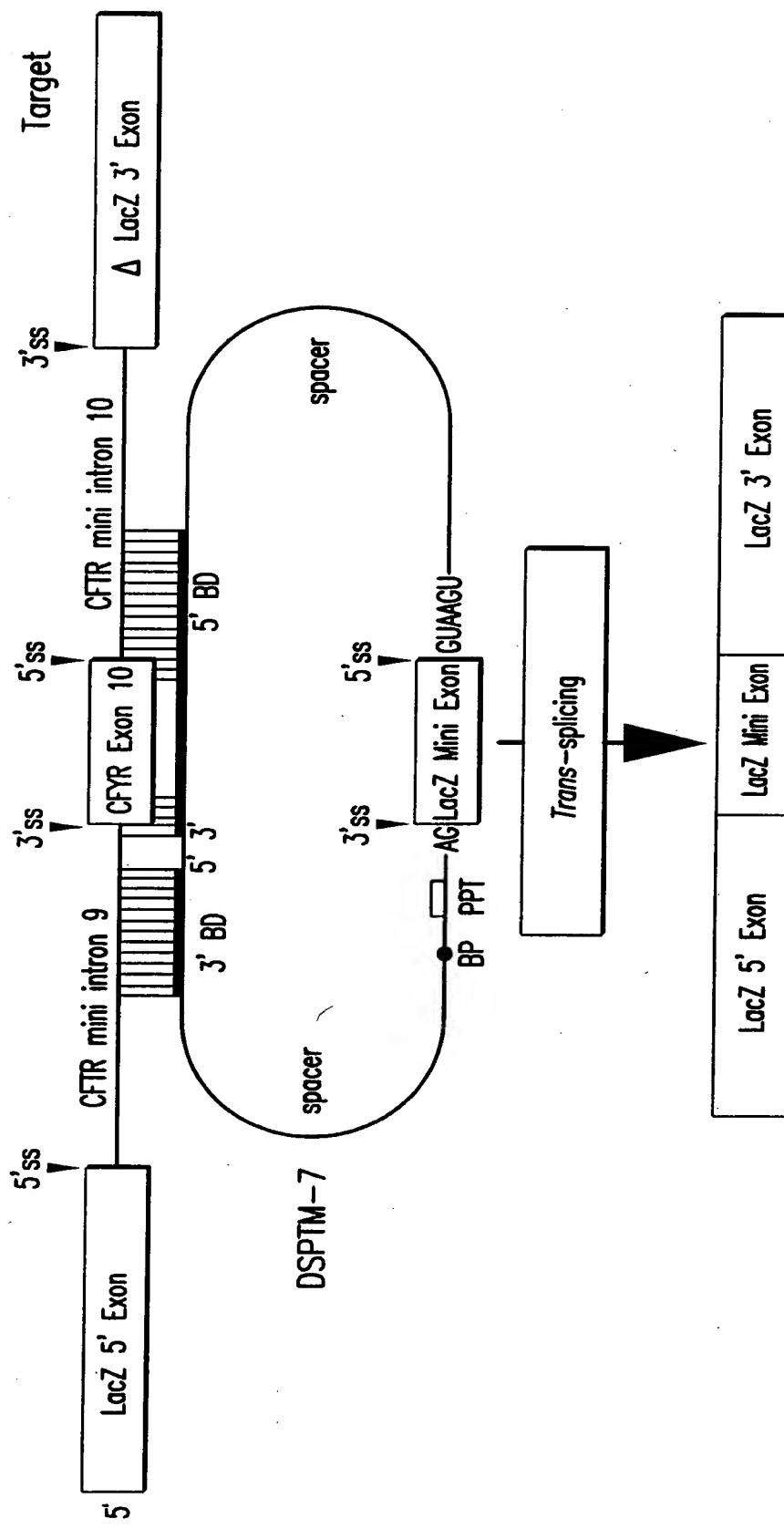
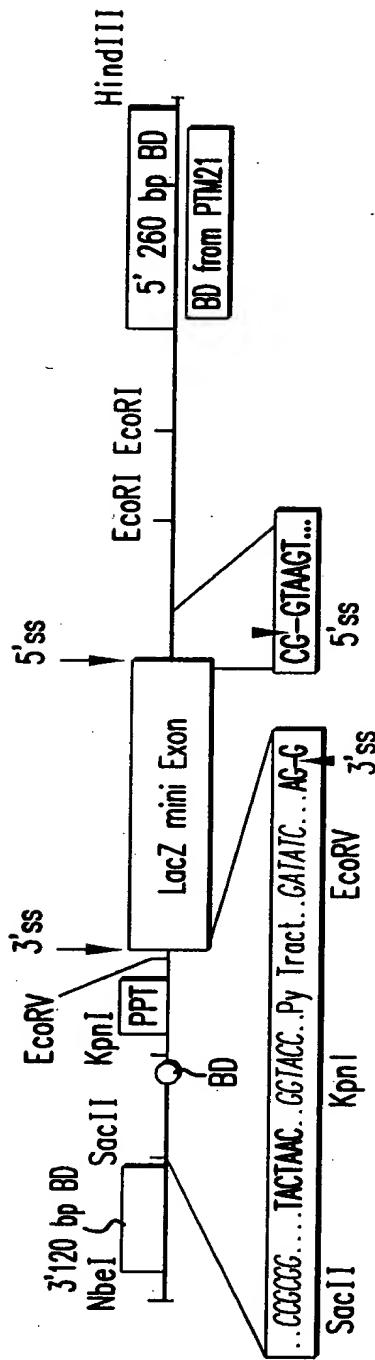


FIG. 20



(1) 3' BD (120 BP): GATTCACTTGCTCAAATTATCATCCCTAAGCAGAAAGTGATACTCTTCTTAAAGATTCTTAAACTCATTGATTC  
AAAAATTAAAATACTCCCTGTTCACTCTGGTACAC

(2) Spacer sequences (24 bp): AACATTATAAACCTGGCTGGAA

(3) Branch point, pyrimidine tract and acceptor splice site: TACTAACCTGGTACCCGATATGTCTAACCTGATTGGGCCCTTGATAAG  
BP            Kpn I            PPT            EcoRV  
LacZ mini 5'ss            exon            3'ss            LacZ mini  
exon

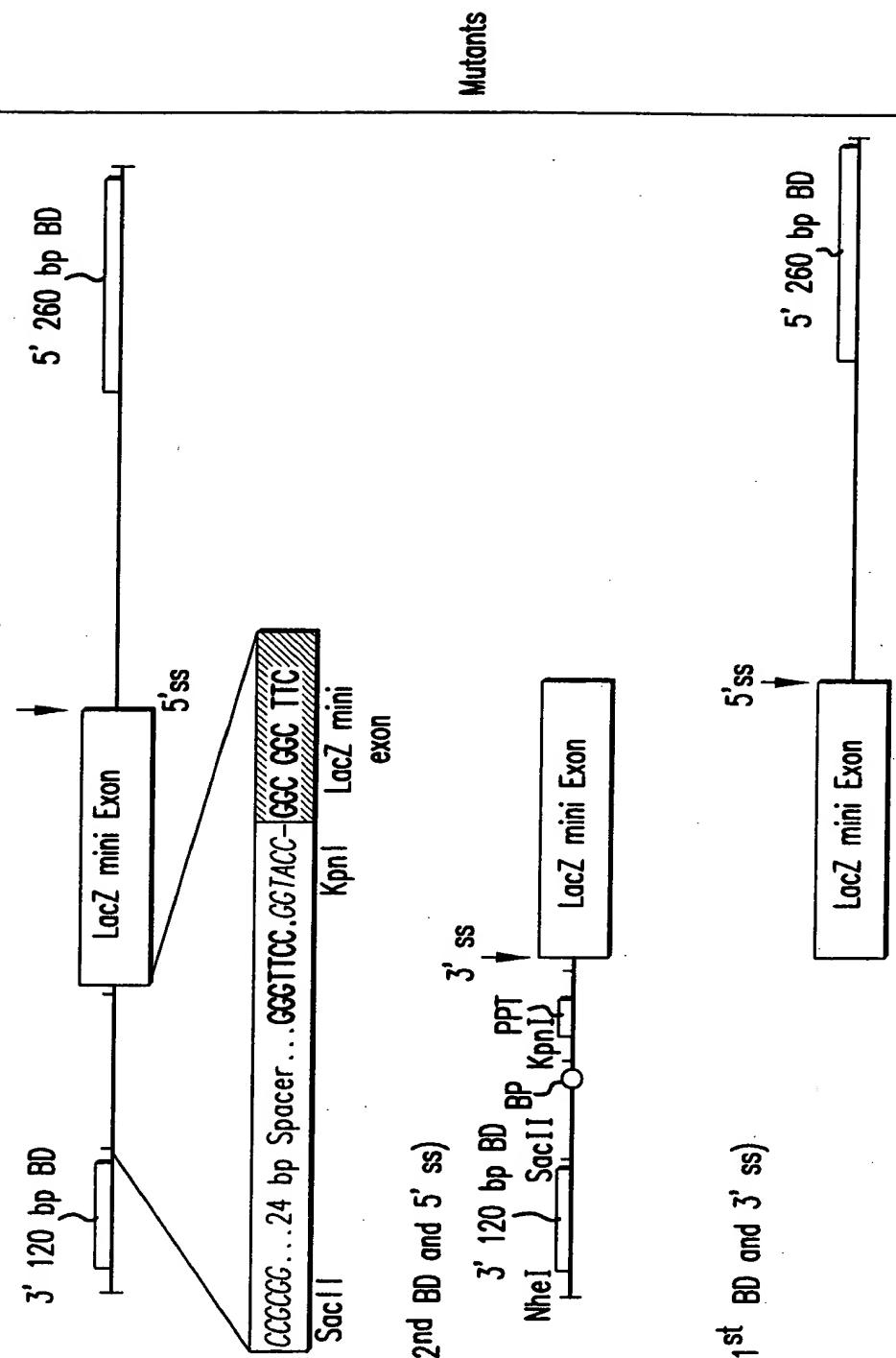
(4) 5' donor site and 2nd spacer sequence: TGA ACG GTAACT GTTATCACCGATATGTCTAACCTGATTGGGCCCTTGATAAG  
CTAAGATCCACOGG

(5) 5' BD (260 BP): TCAAAAAGTTTTCACATAATTCTTACCTCTCTTCAATTCTATGACGGCTTCATGACGGAA  
ACACCAATGATTTTCTTAACTGGCTGCCATAATTCTGGAAACTGATAACACAACTGAATTCTTCACTGTGCTAA  
AAAAACCCCTCTGAATTCTCCATTTCCTCCATAATCATCATACACTGAAATAAAACCCATCAATTAACTCA  
TTATCAAATCACCG

**FIG. 21**



**DSPTM8:** ( $\Delta$  3' ss; 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



**FIG.22**



### ACCURACY OF DOUBLE TRANS-SPlicing REACTION

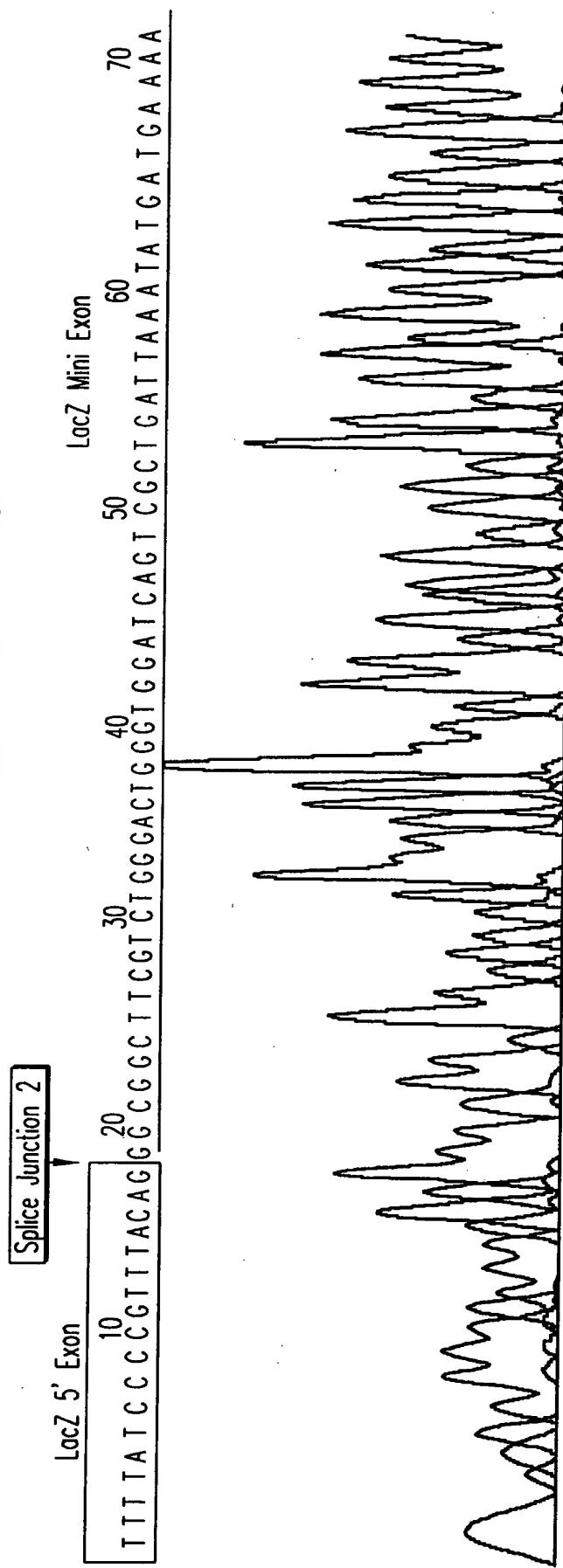


FIG. 23A



ACCURACY OF DOUBLE TRANS-SPLICING REACTION

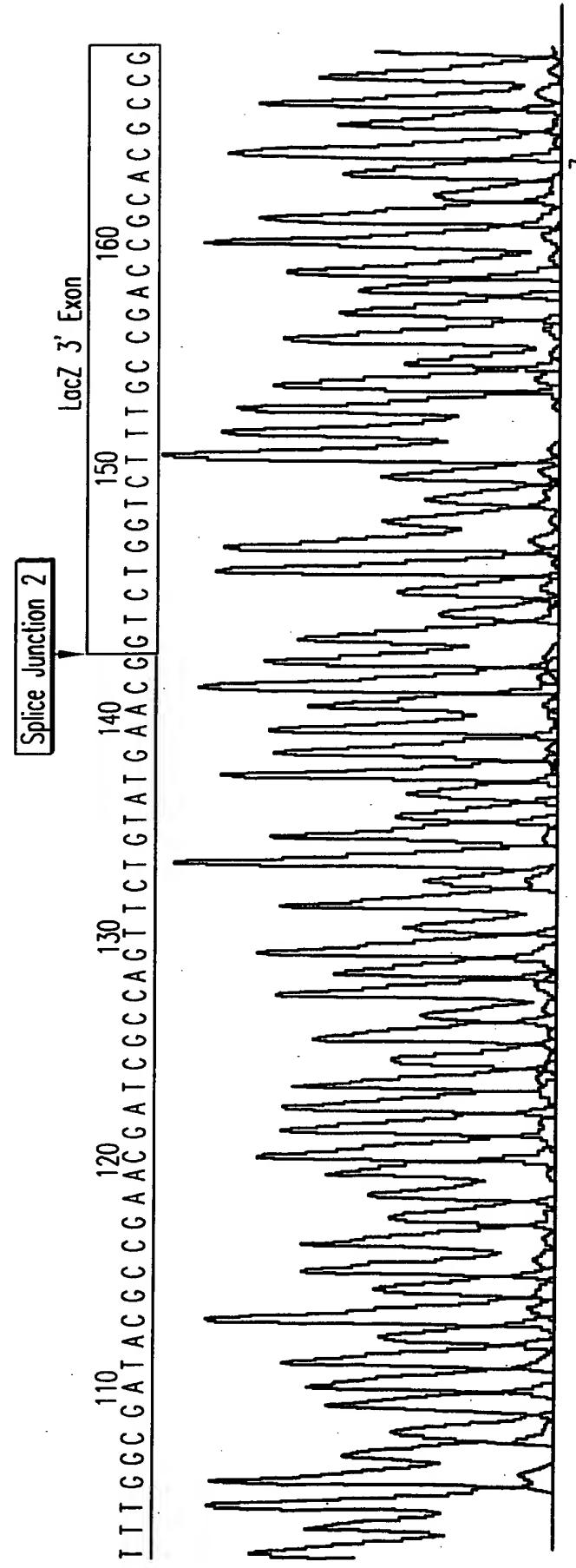


FIG. 23B



### Double Trans-splicing Produces Full-length Protein

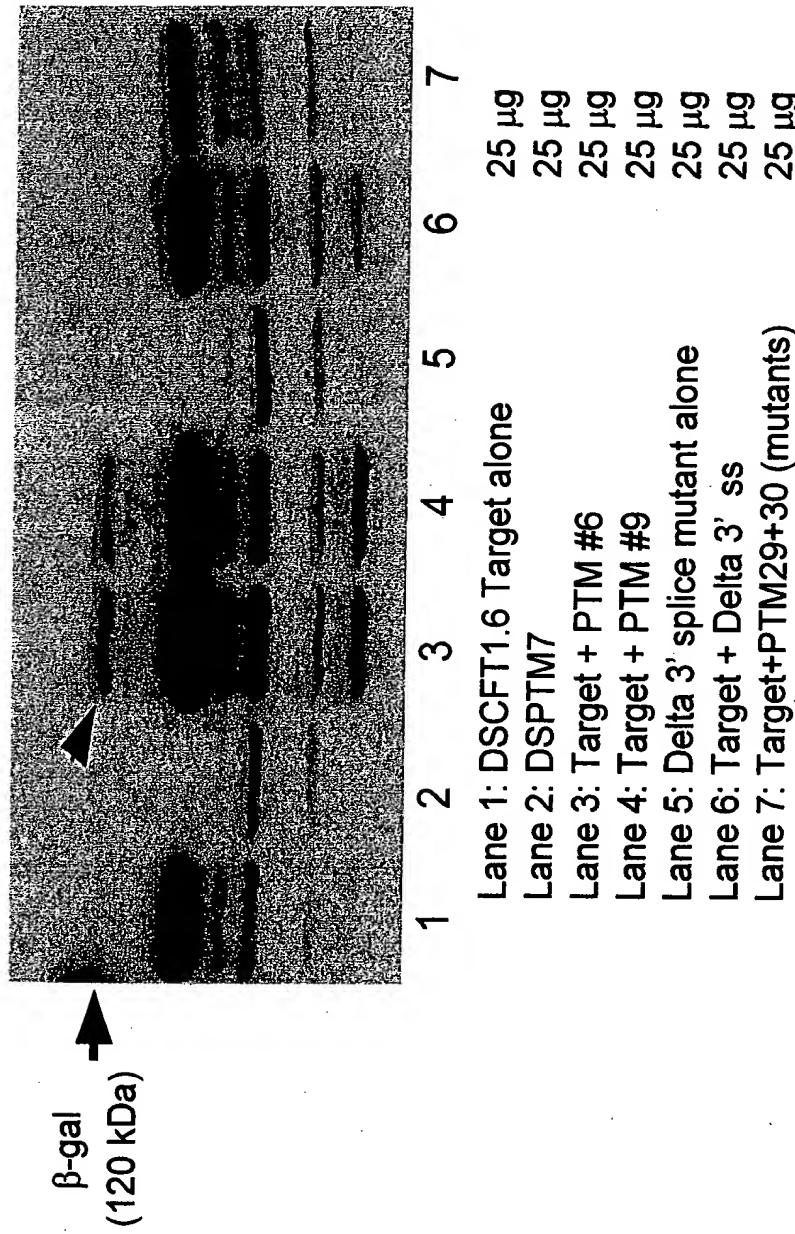


FIG. 24

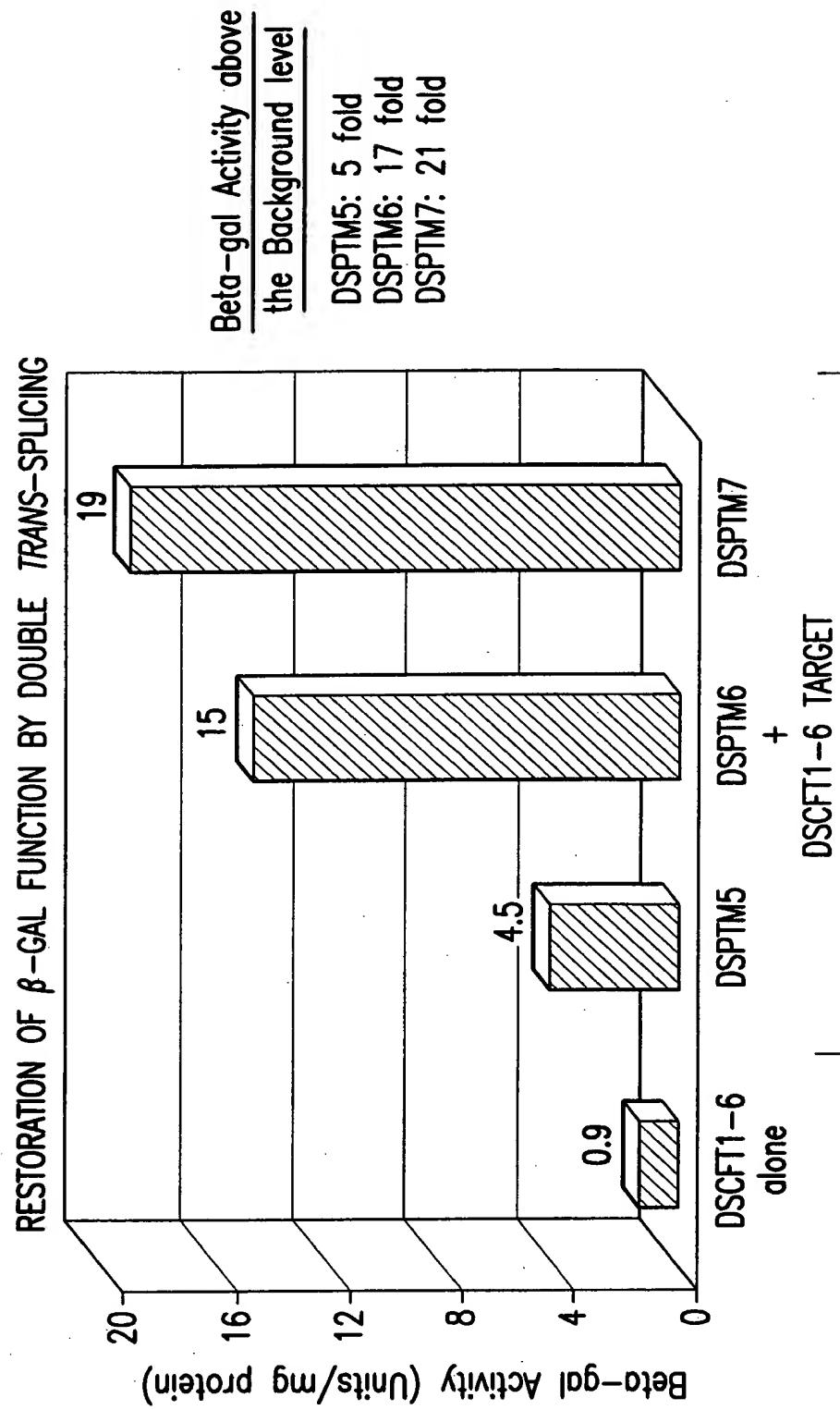
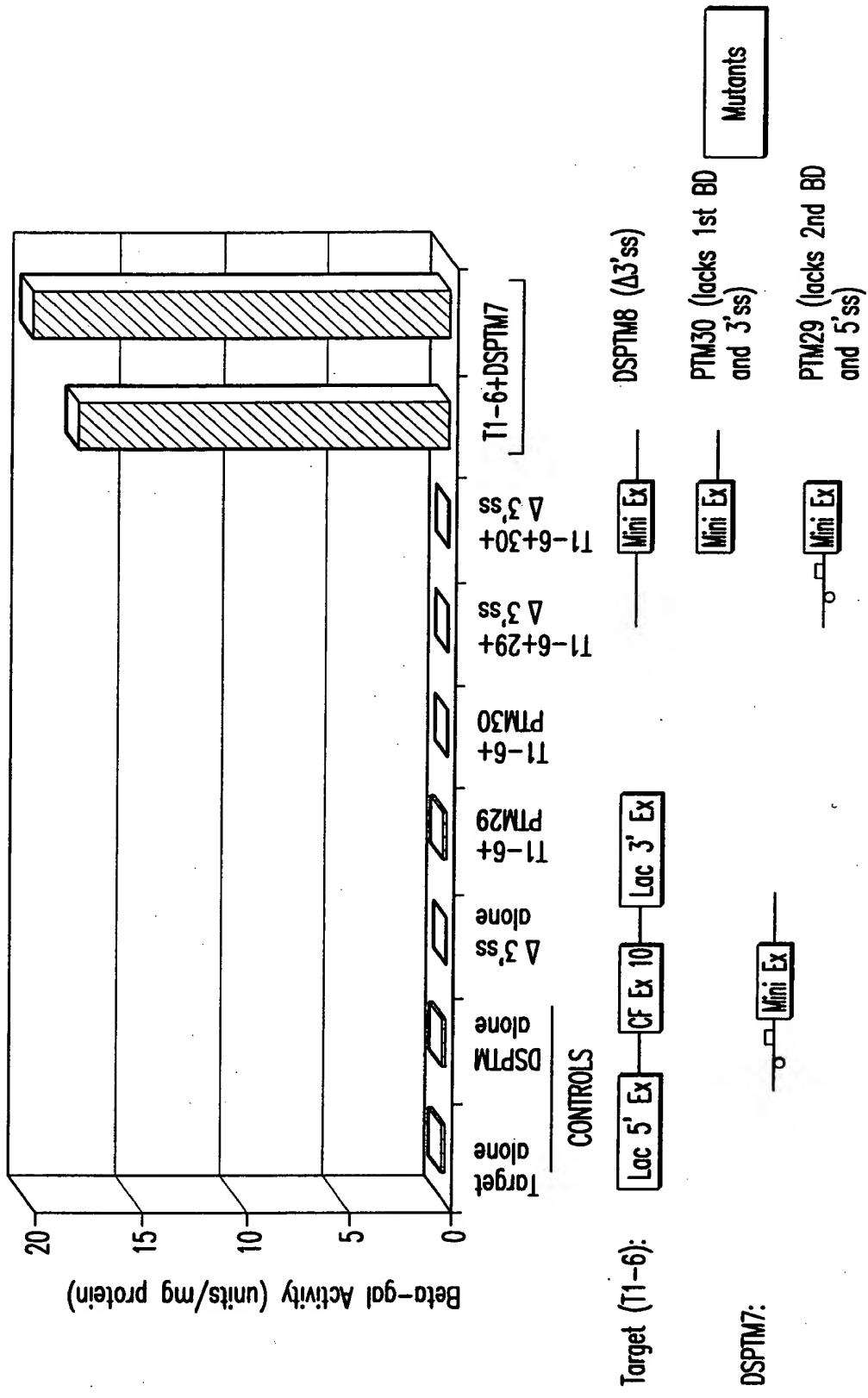


FIG.25

IPM  
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EXCISE & TRADE

**RESTORATION OF  $\beta$ -GAL ACTIVITY IS DUE TO DOUBLE RNA  
TRANS-SPlicing EVENTS**



**FIG. 26**



### DOUBLE TRANS-SPlicing: TITRATION OF TARGET & PTM

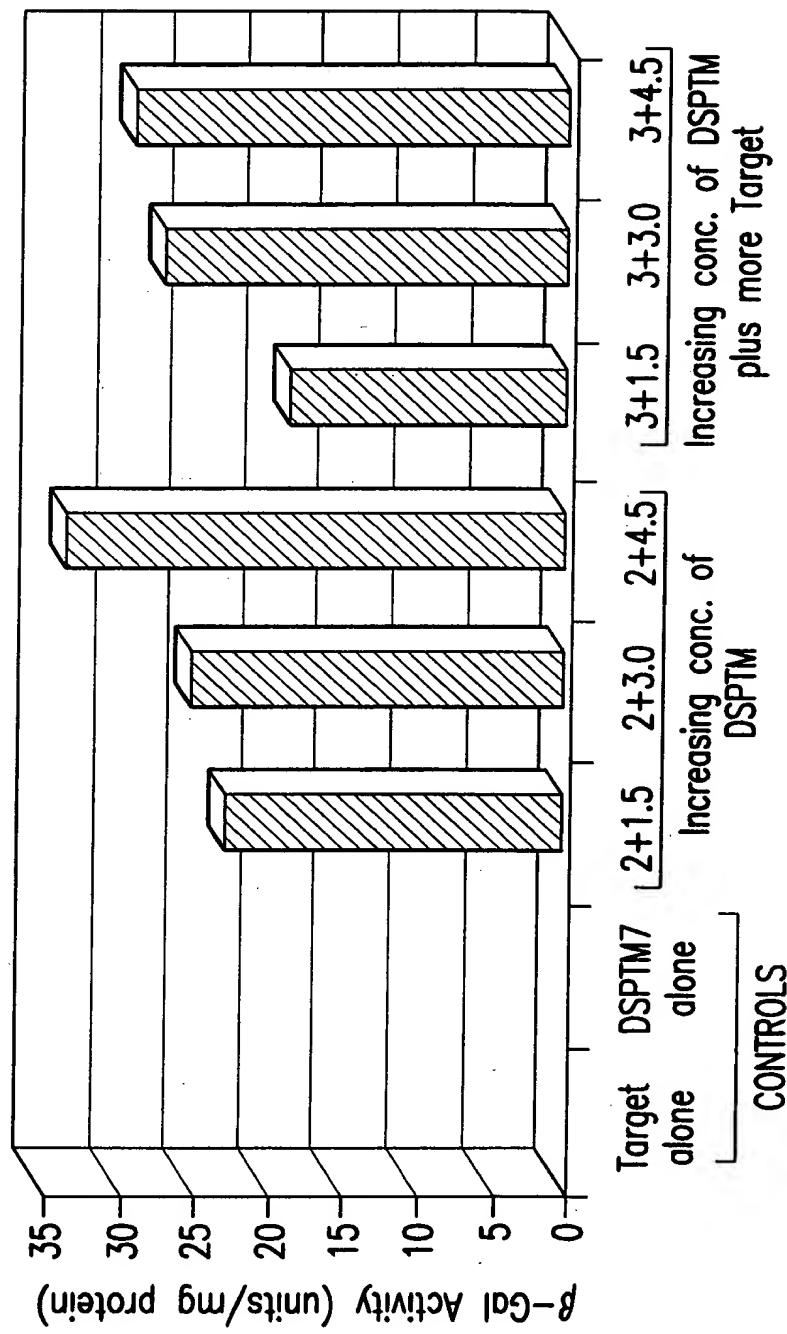


FIG.27

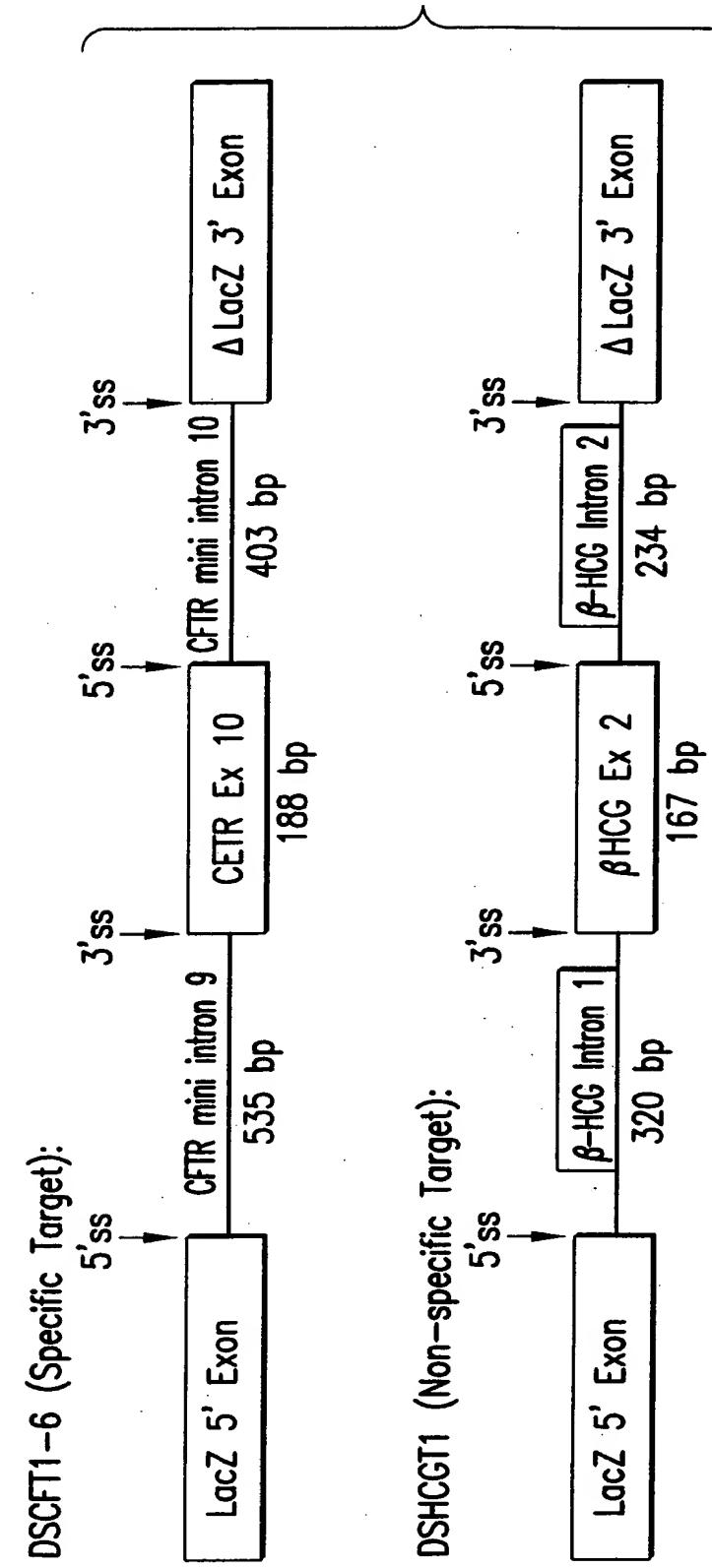


FIG. 28



### SPECIFICITY OF DOUBLE TRANS-SPlicing REACTION

34

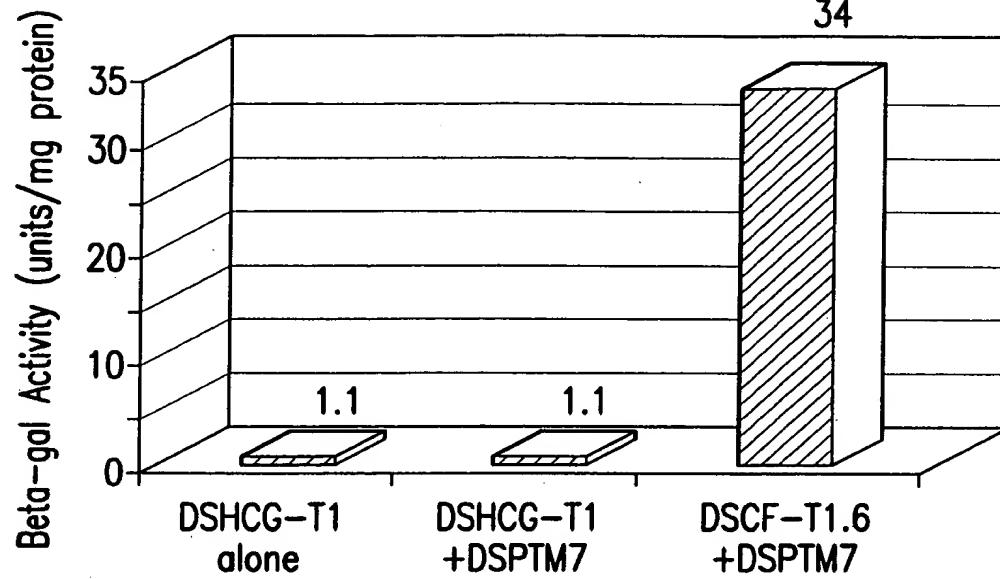


FIG.29

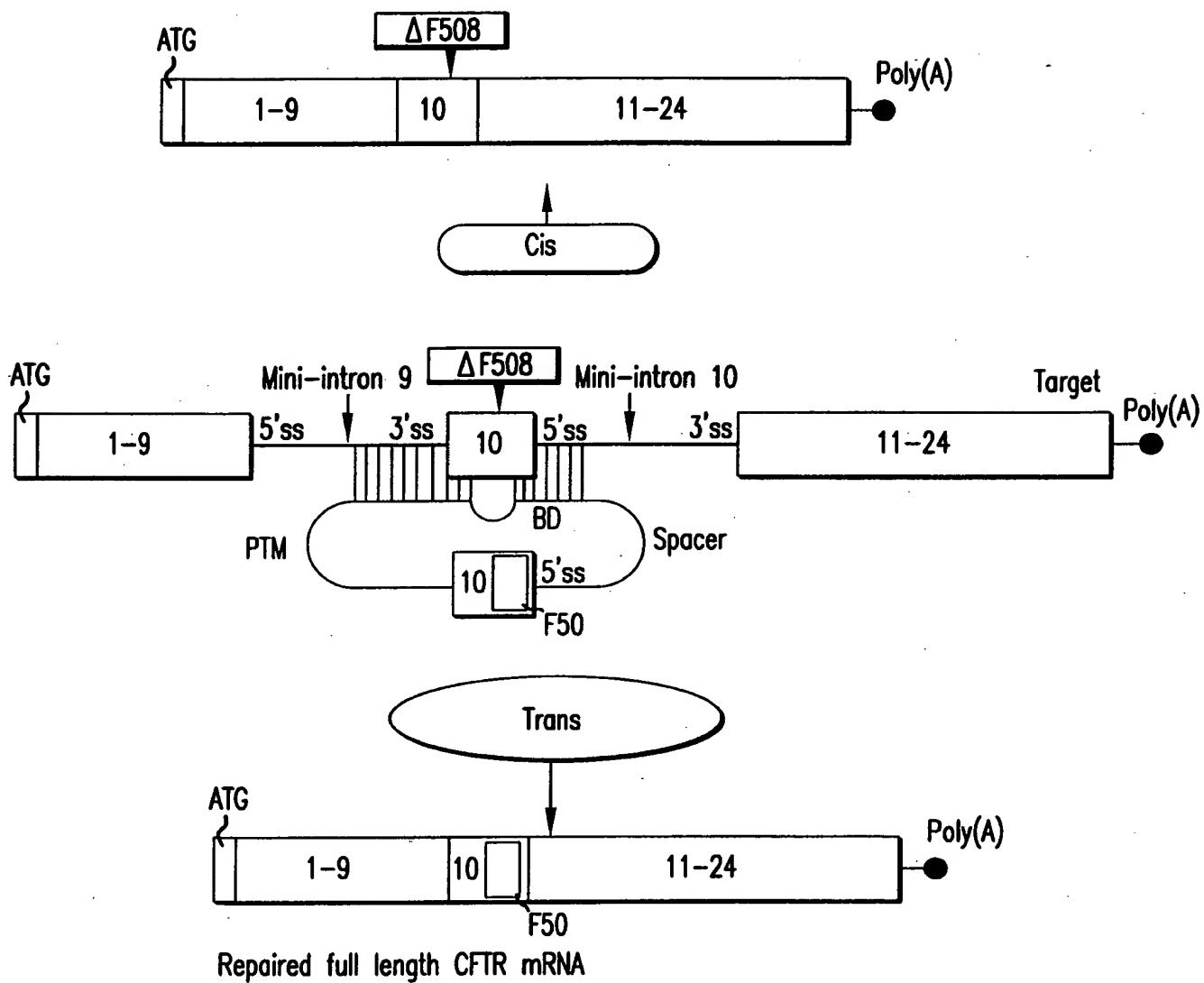
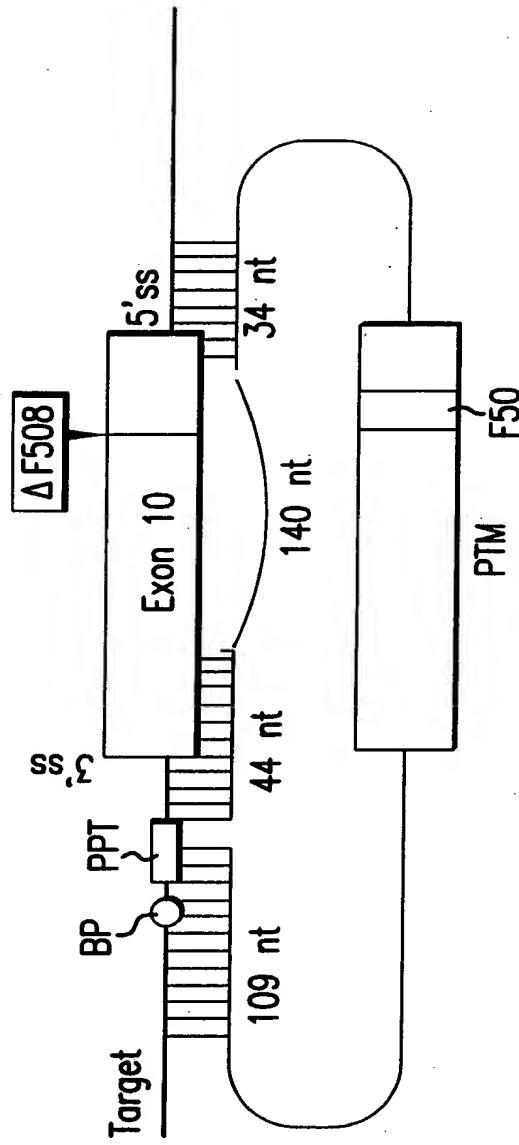


FIG.30



PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target



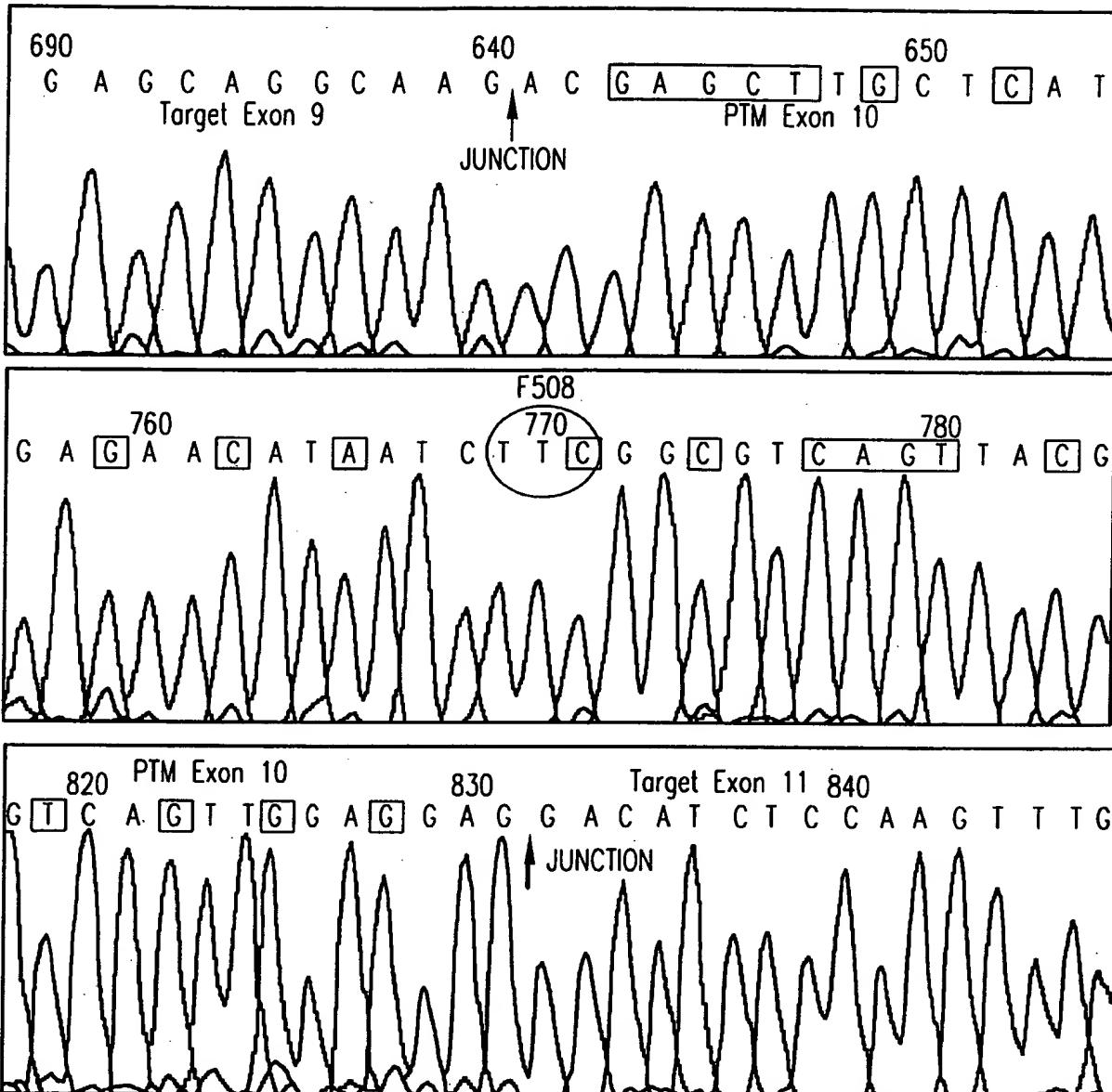
ACAGCTTGC**T**CATGATCATGGCCAGT TAGAACCCAAGTGAGGCAAGTCAAACATTCCCG  
GGCCCATCAGCTTTGGCCAATTTCAGTGGCATTCATGGCCCCGTACCATCAGGGAGAACAAAT  
C7TGGCCGTCAGTTACCCGAGTACCCGTAGGCCTATCGCCTGGTGATTAAGCCGGTCAGTGGAGGAC

MCU in exon 10 of PTM  
88 Of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

FIG. 31



Sequence of a double  
Trans-spliced product



[ ] = MCU in  
PTM exon 10

FIG.32



CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splices site of intron 10 of a mini-gene target

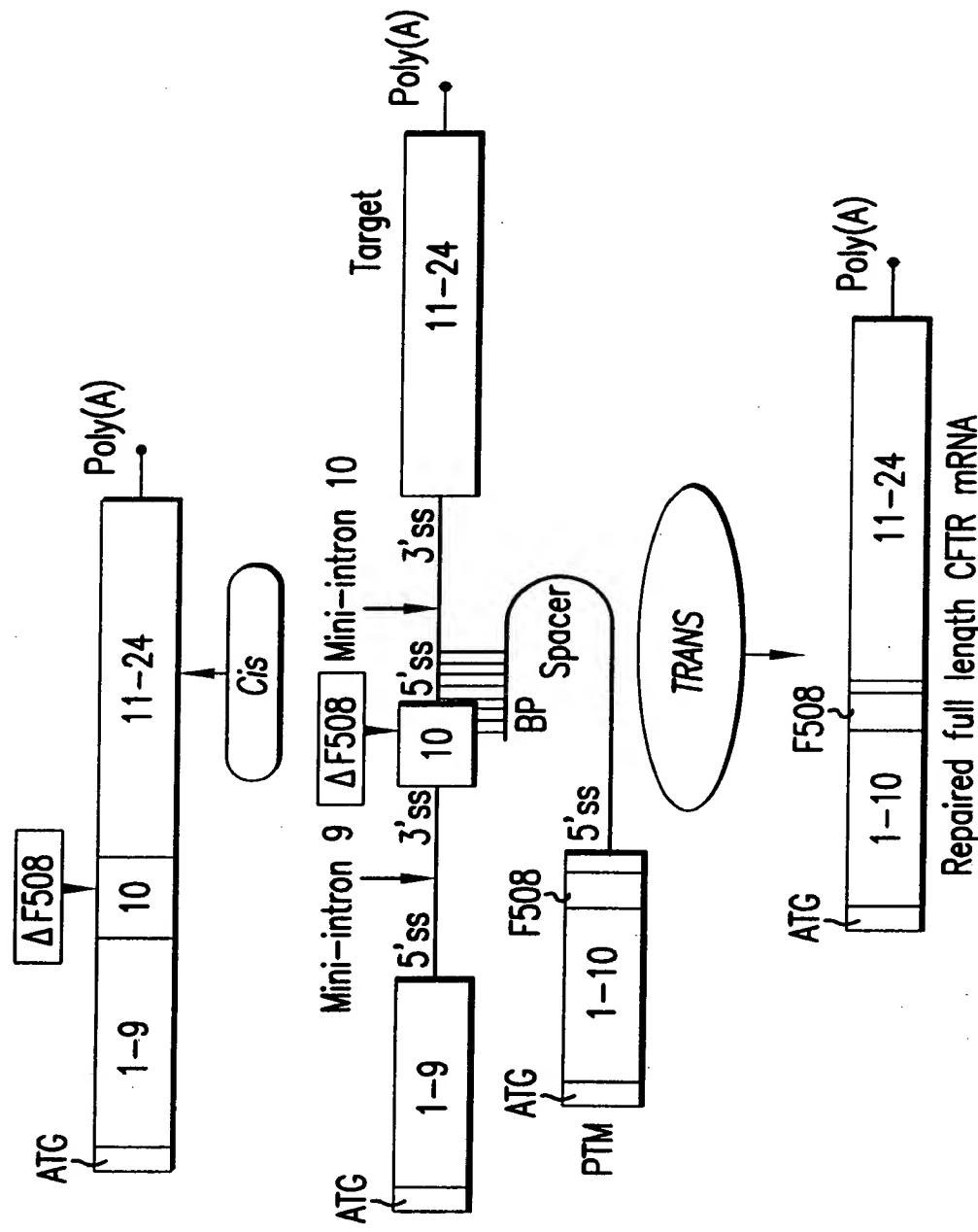


FIG.33



PTM with a short binding domain masking a single splice site in a mini-gene target.

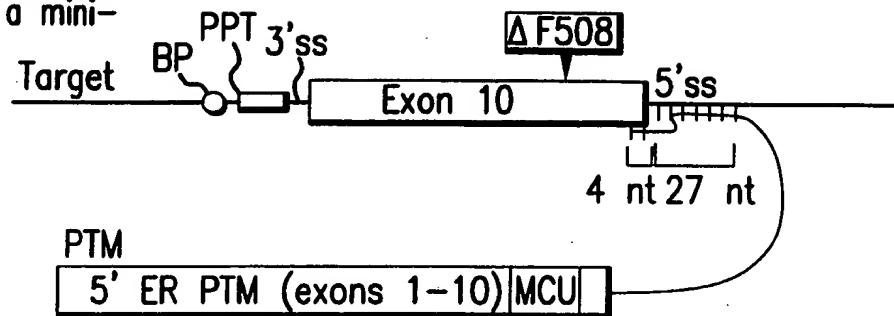


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

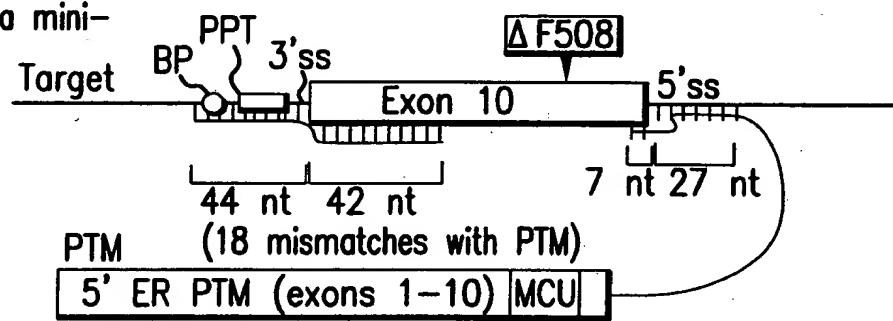


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

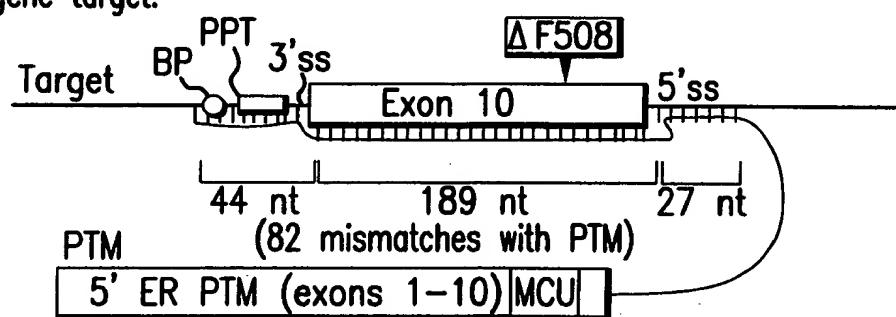


FIG.34C

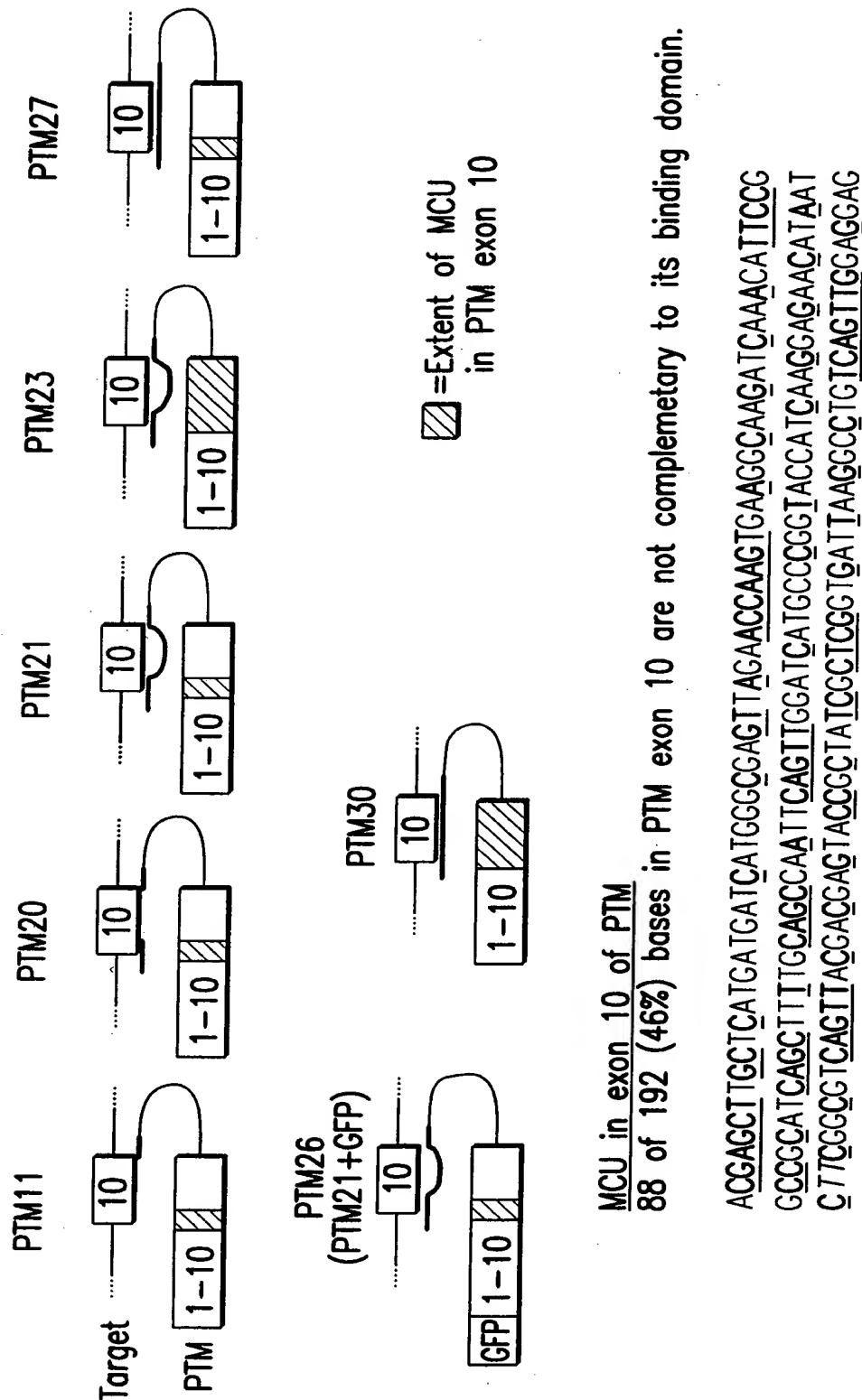
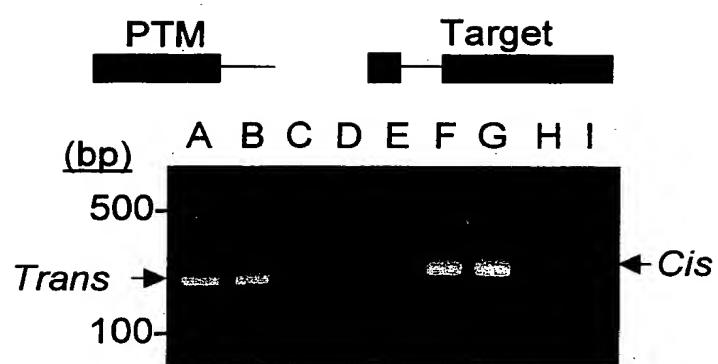


FIG.35



**FIG. 36A**



*Cis-spliced product*  
[Primers CF1+CF111]

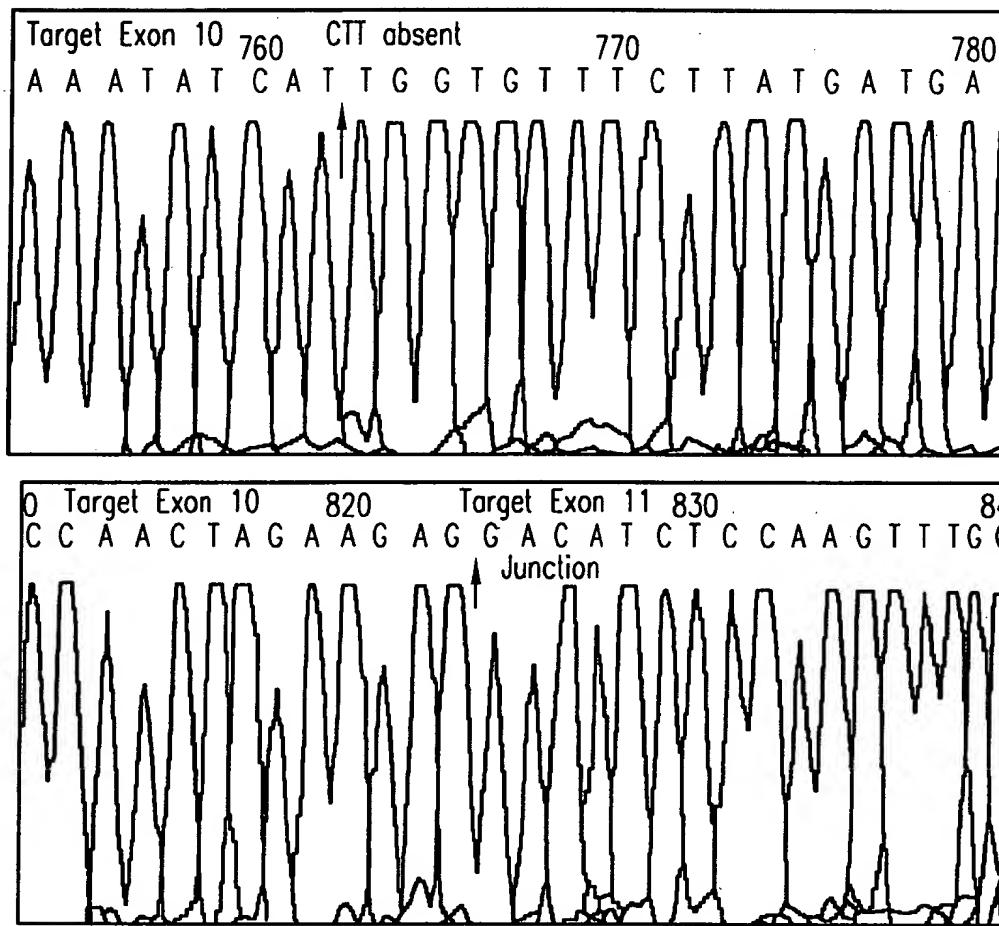


FIG.36A-1

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*Trans-spliced product*  
[Primers CF93+CF111]

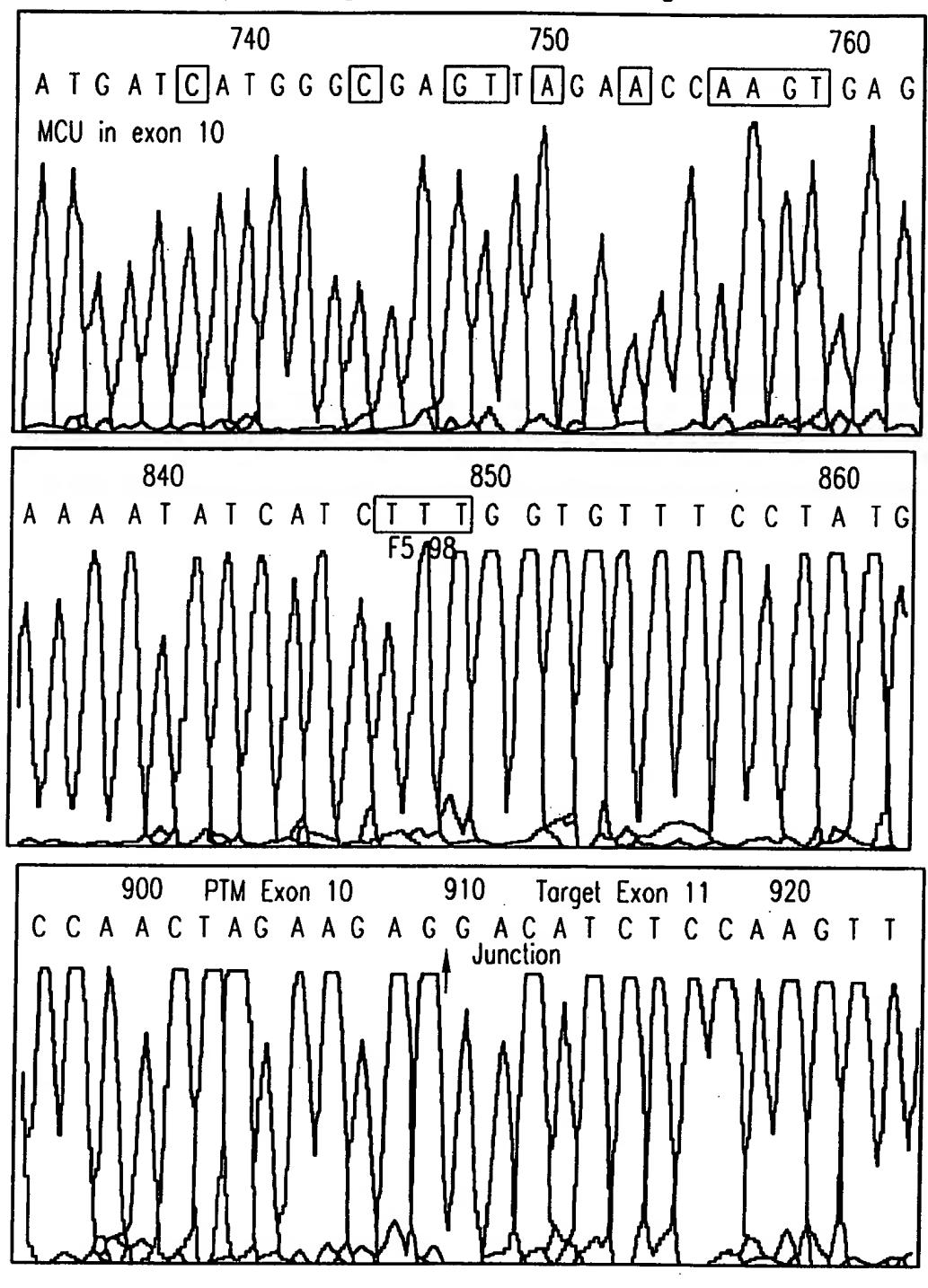


FIG.36B

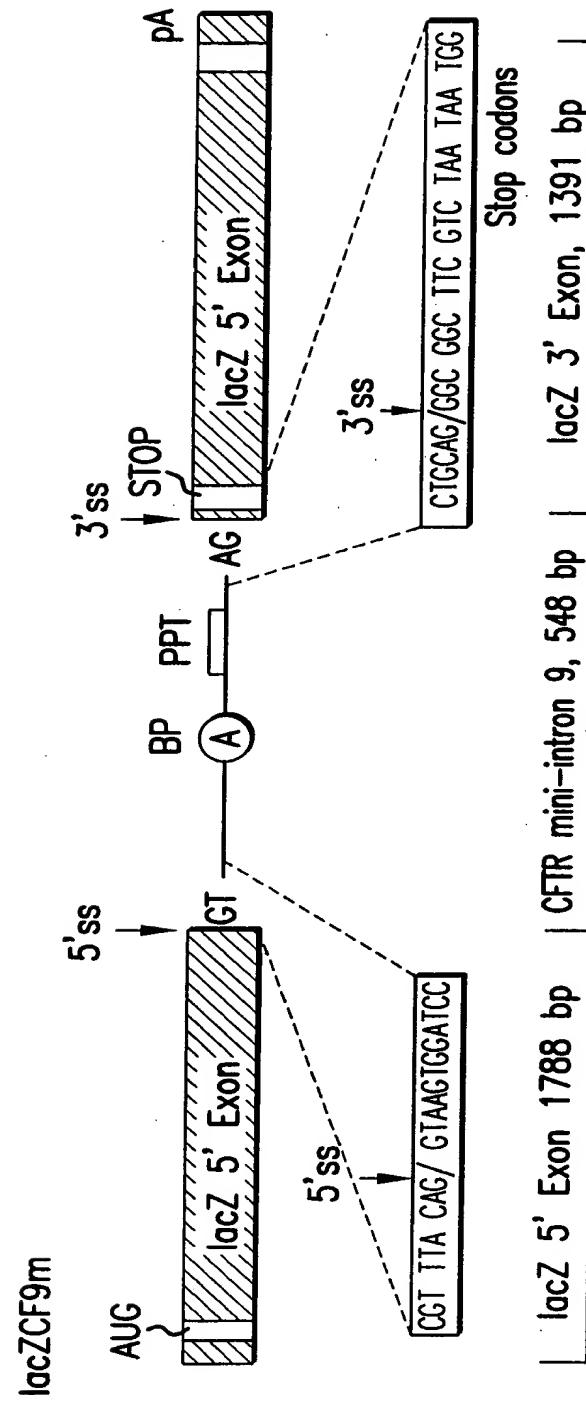


FIG. 37A

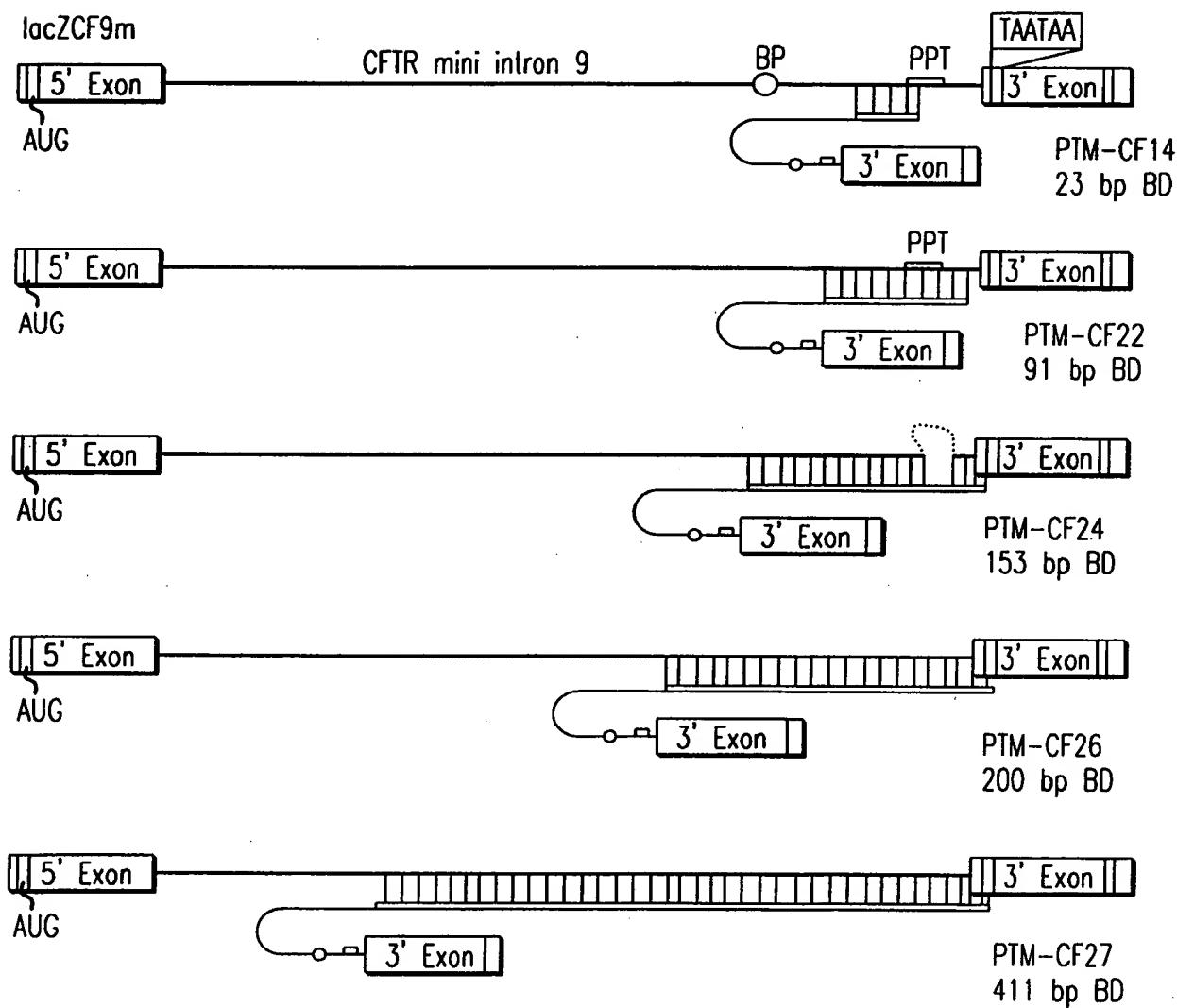
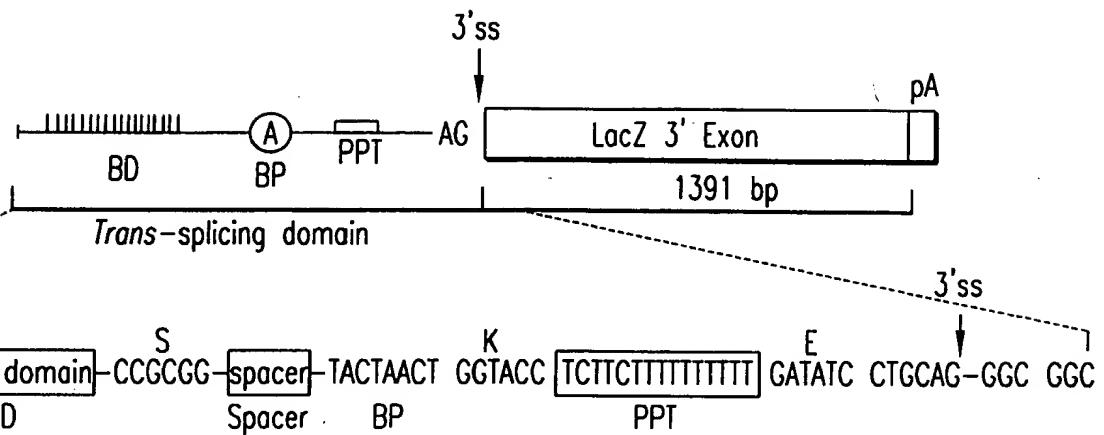


FIG.37B

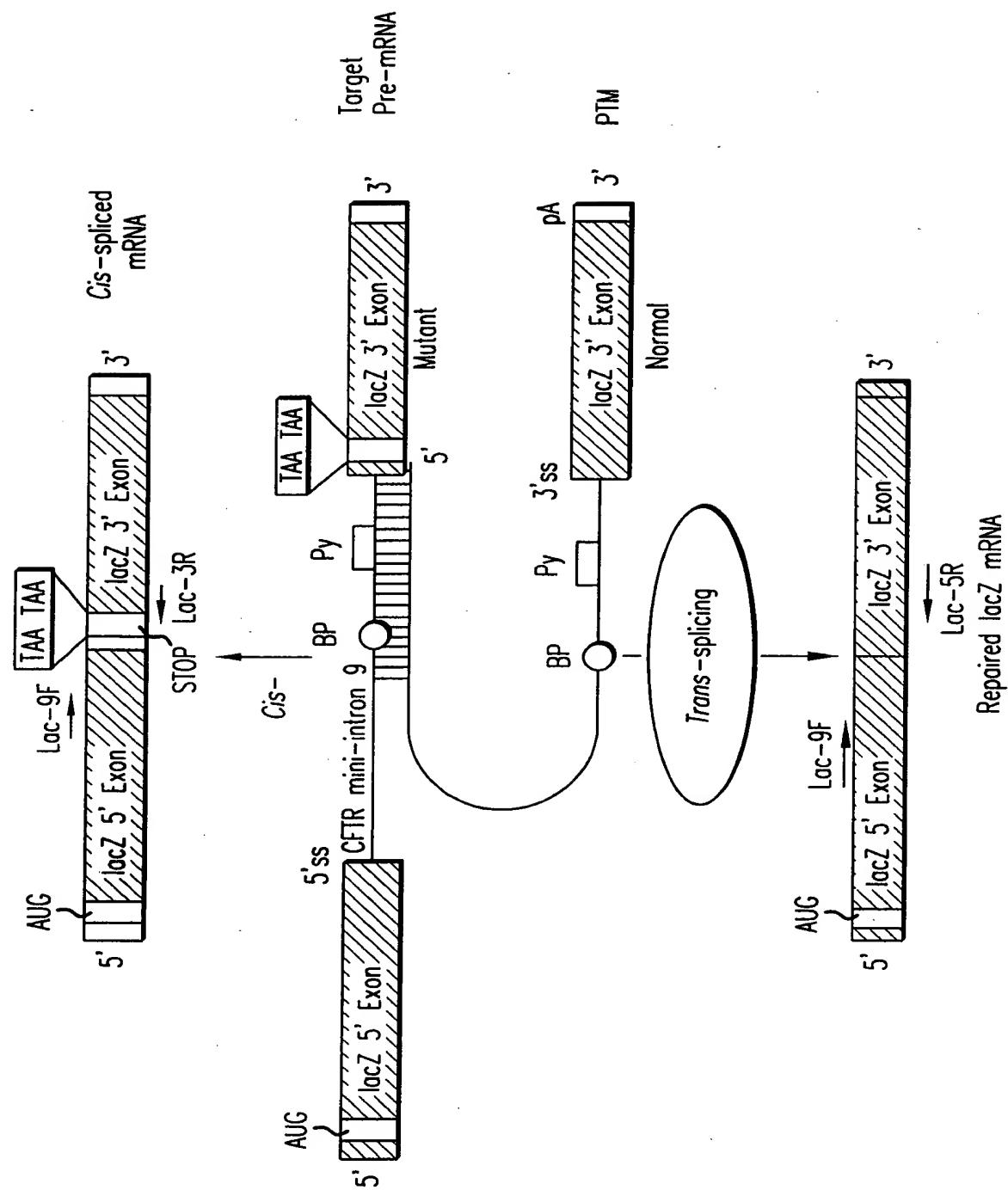


FIG. 37C

O I P M  
NOV 24 2008  
EXHIBIT & TRAILER

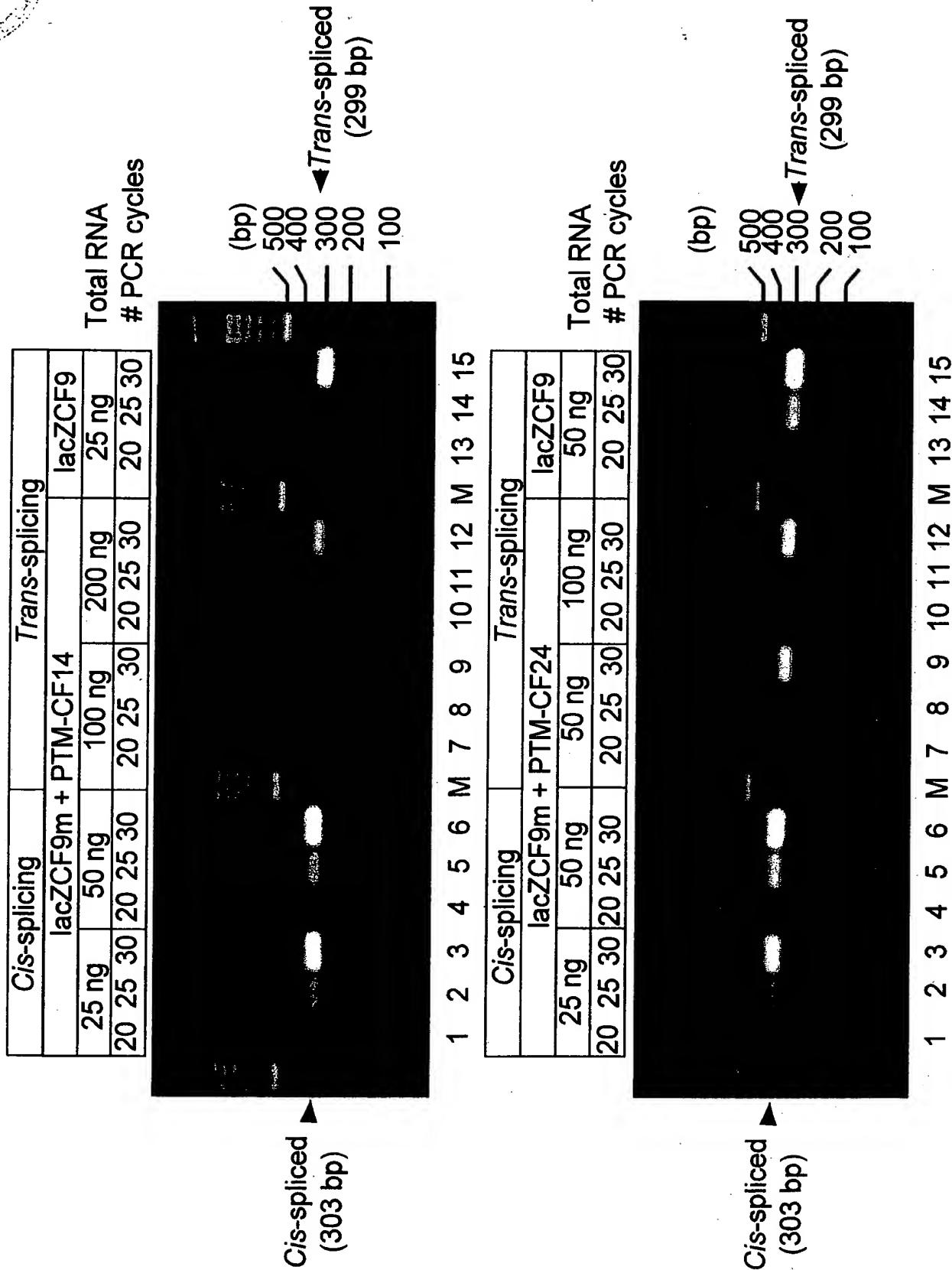


FIG.38A

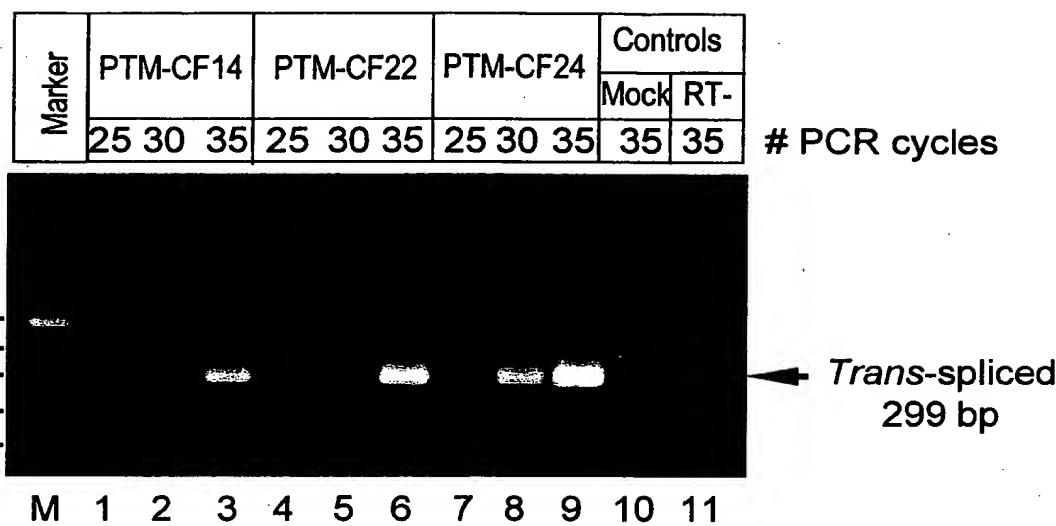


FIG.38B

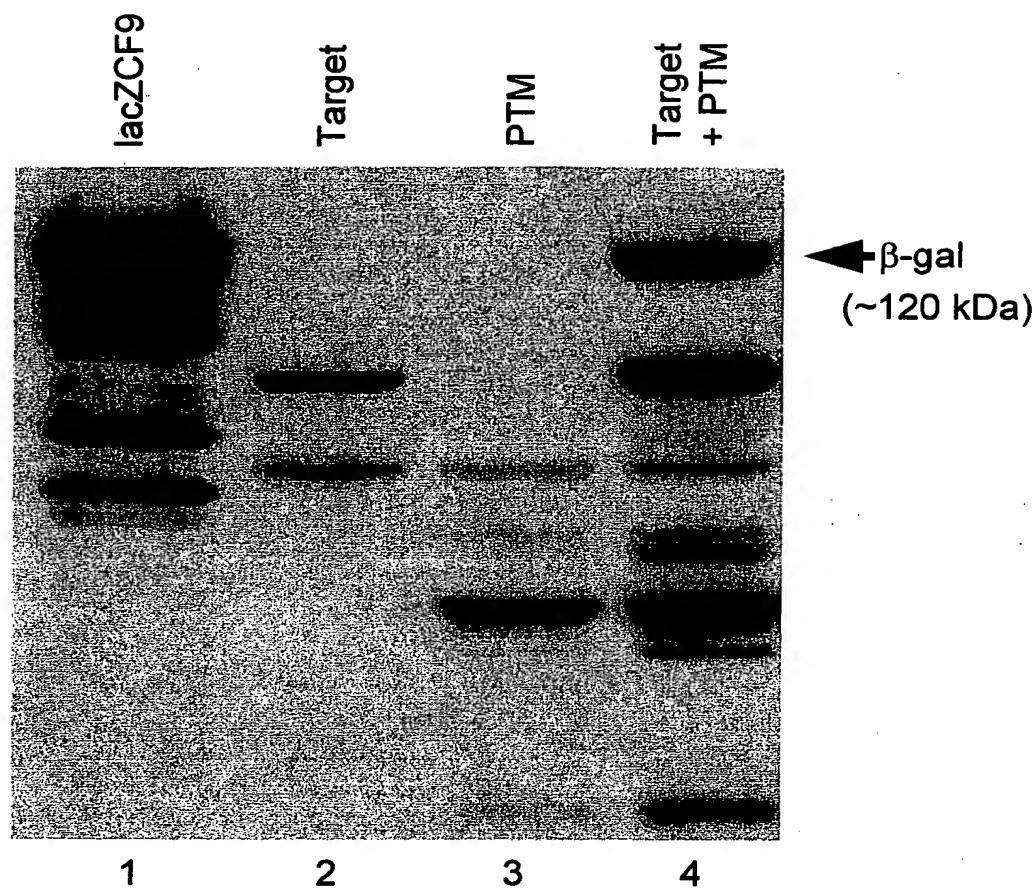
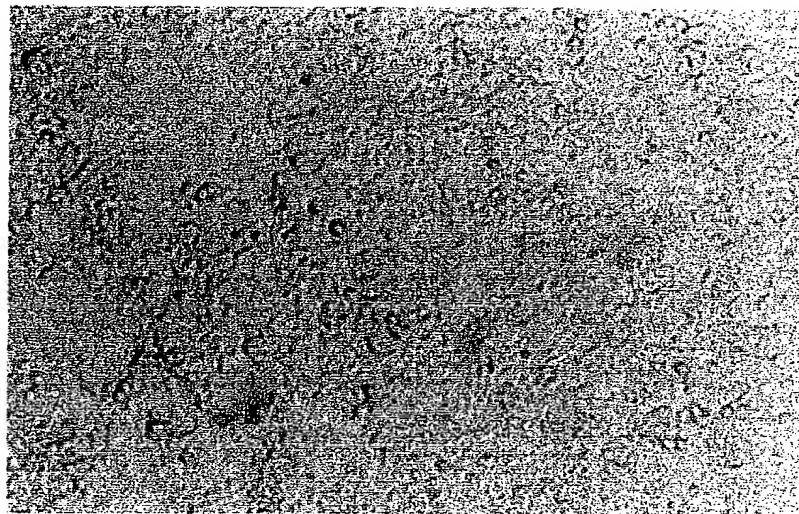
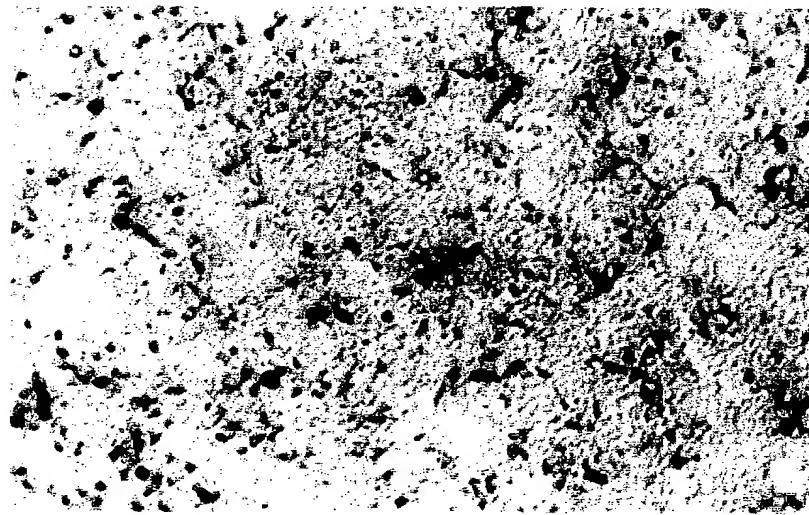


FIG.39



**FIG.40A(a)**



**FIG.40A(b)**

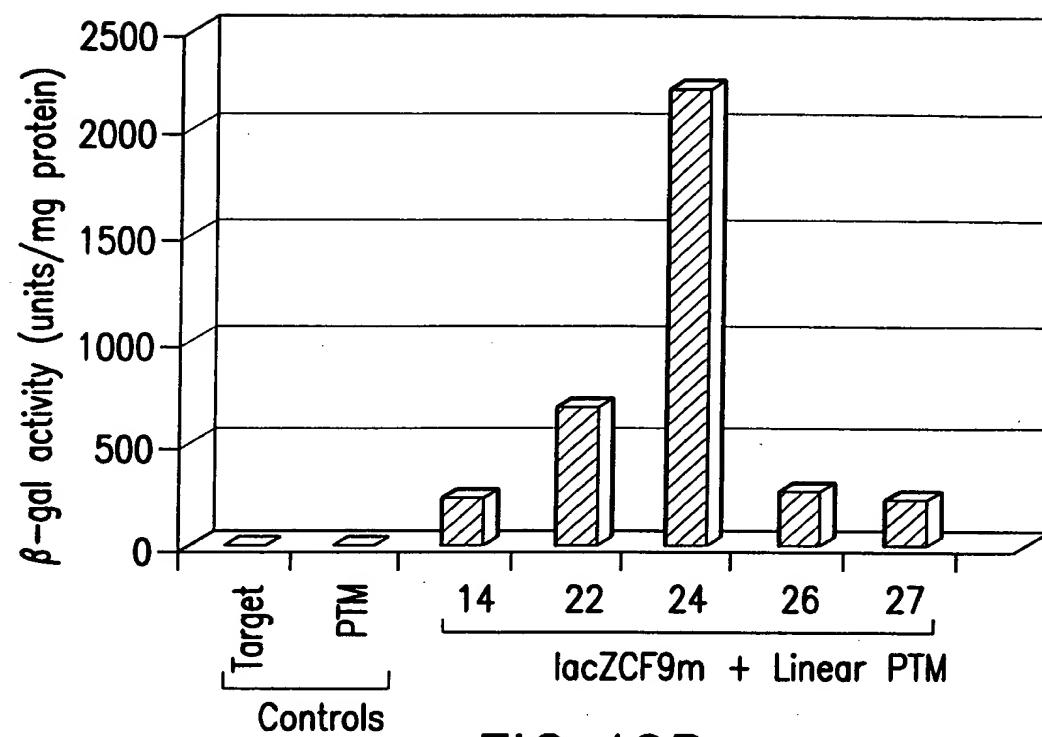


FIG.40B

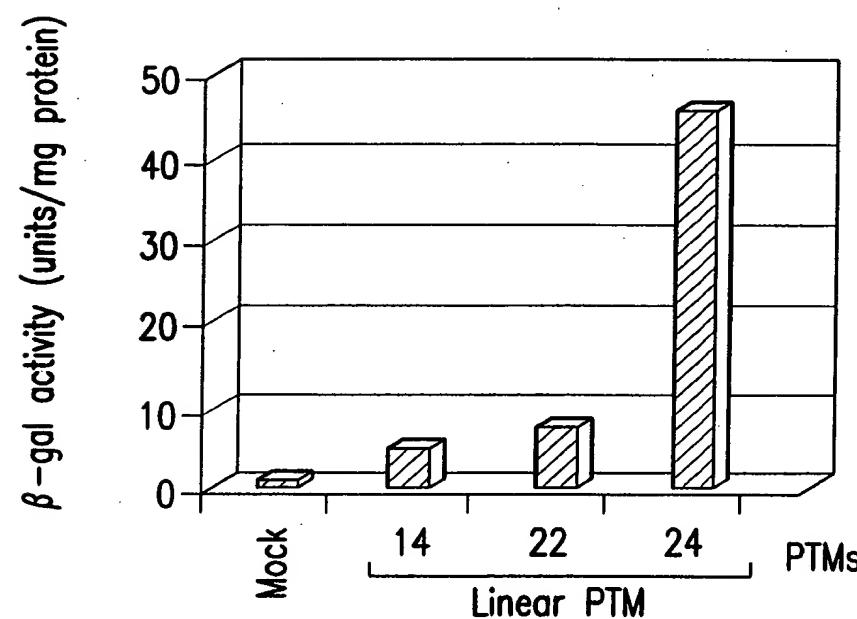


FIG.40C

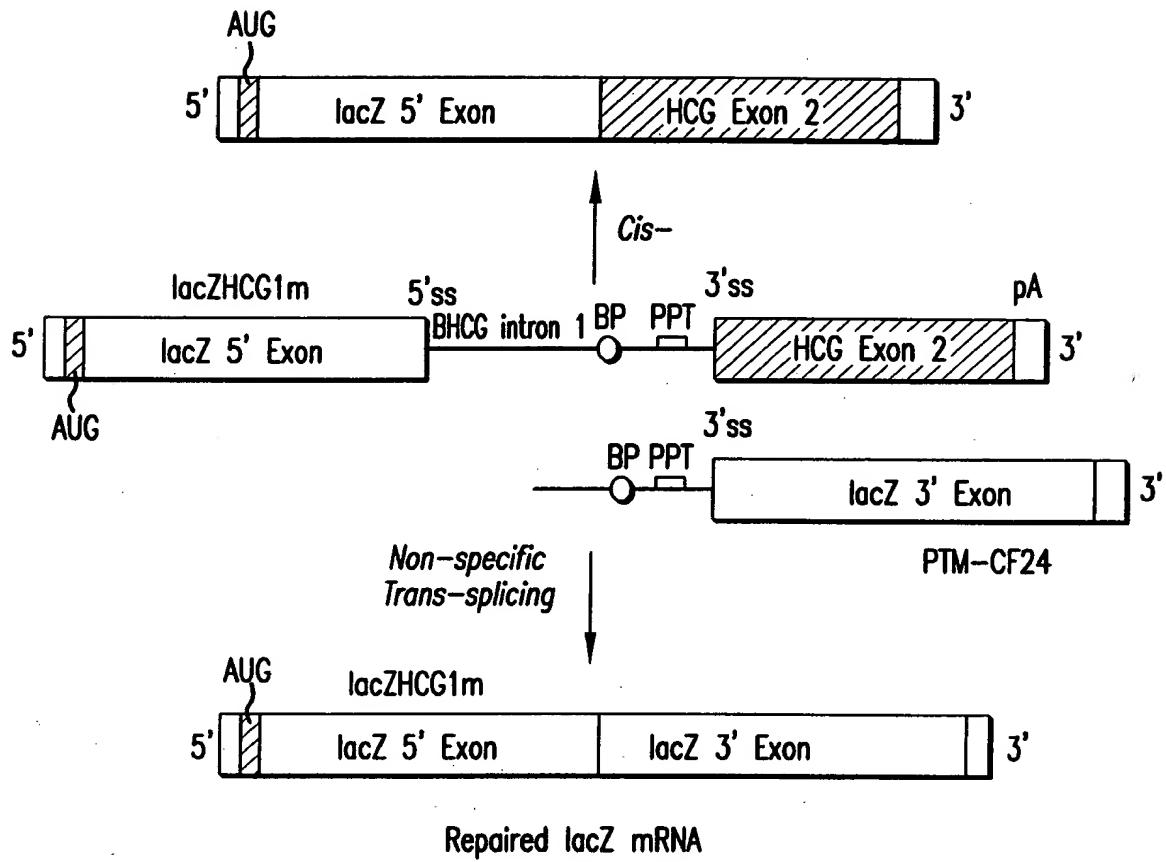


FIG.41A

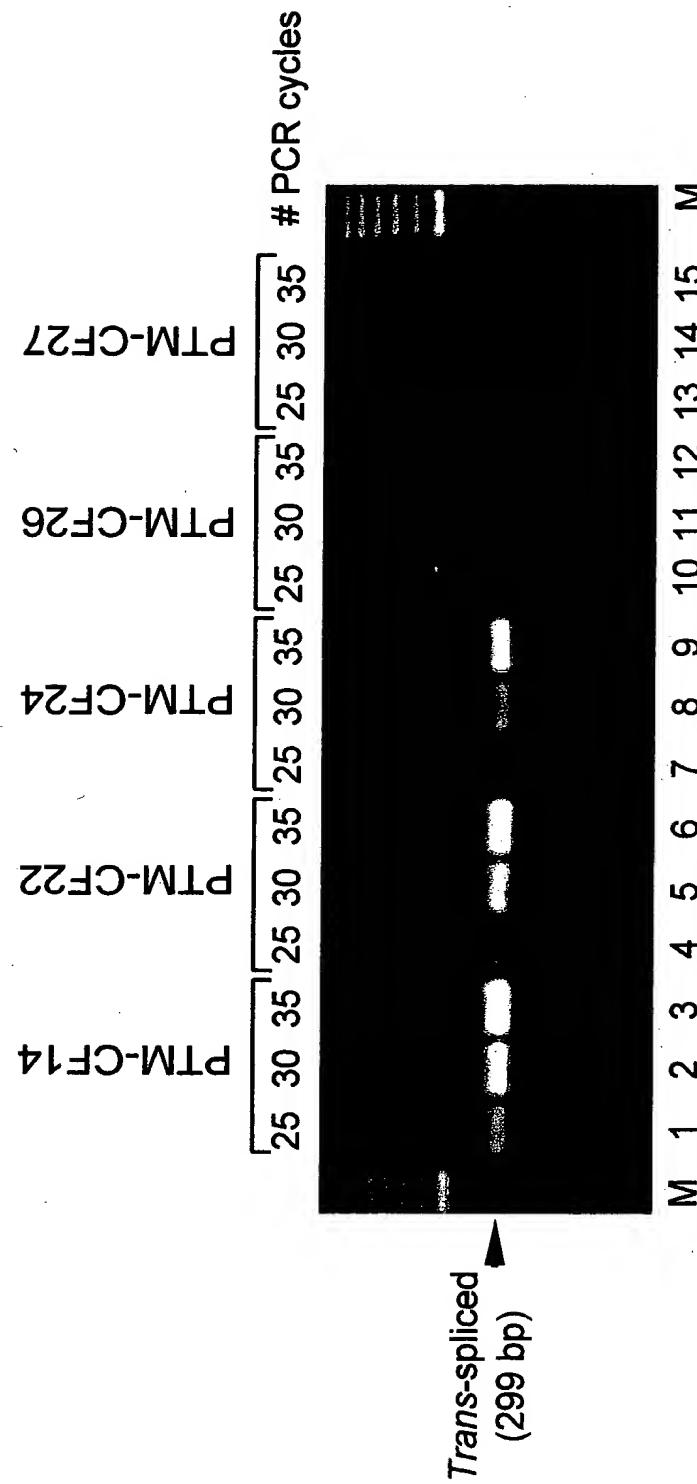


FIG. 41B

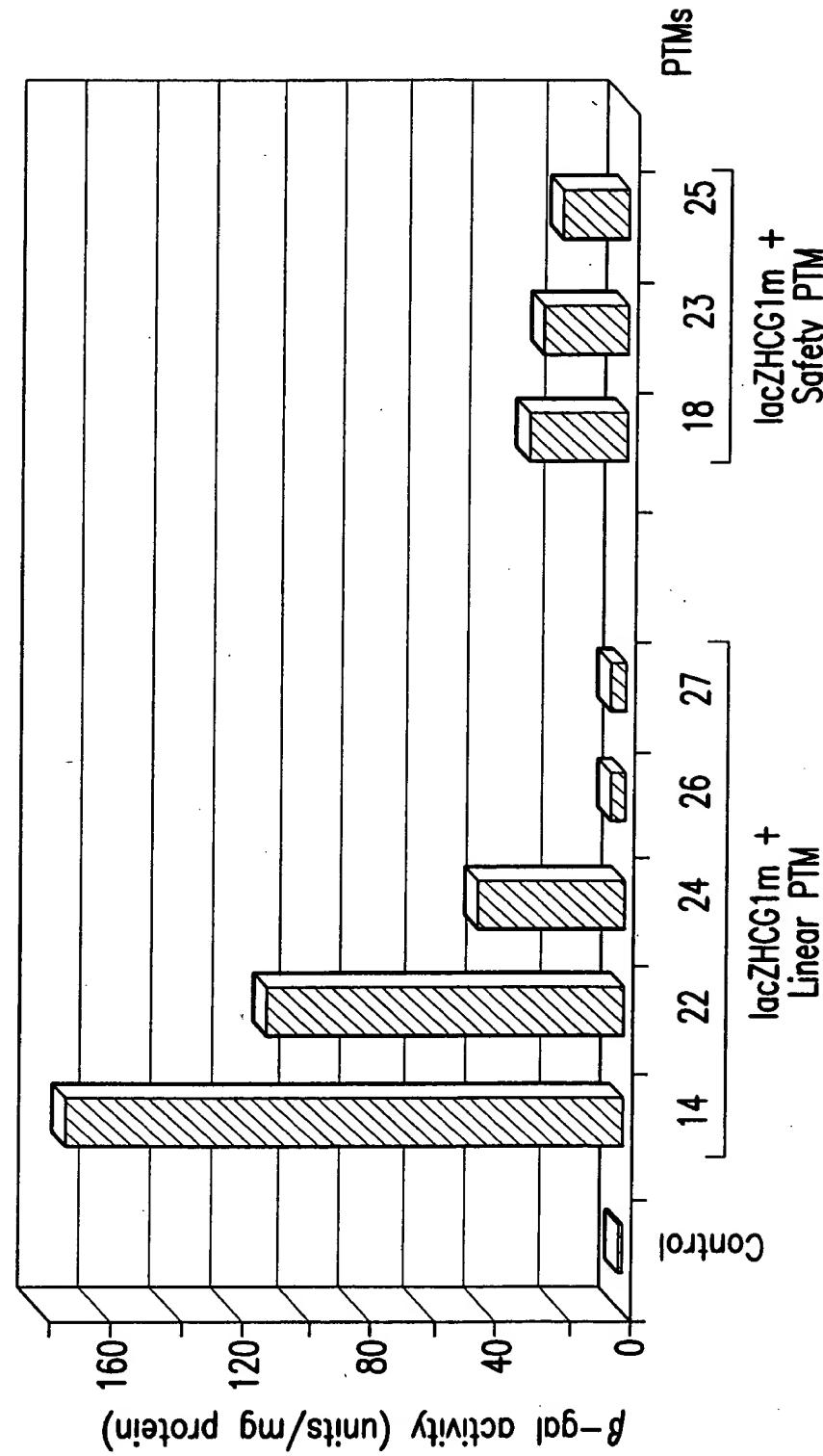


FIG. 41C



Exons

1-10

ATGCAGAGTCGCCCTGGAAAAGGCCAGCGTTGTCCAAACTTTTCACTGGACCAGACCAATTGAGGAAC  
GATACAGACAGCCCTGGAATTGTCAGACATACCAAATCCCTCTGTTGATTCTGTCACAATCTATCTGAAAATT  
GGAAAGAGAATGGGATAGAGAGCTGGCTCAAAGAAAAACTCTAAACTCATTAATGCCCTCGGGATTTTCTGG  
AGATTTATGTTCTATGGAATCTTTATTTAGGGAACTACCAAAGCAGTACAGCCTCTTTACTGGGAAGAATCA  
TAGCTCCTATGACCCGATAACAAGGAGAACGCTCTATCGCGATTATCTAGGCATAGGTTATGCCCTCTTTAT  
TGTGAGGACACTGCTCCTACACCCAGCCATTGGCCTTCATCACATTGGAATGCCAGATGAGAATAGCTATGTTAGT  
TTGATTATAAGAACACTTAAAGCTGCAAGCGTTCTAGATAAAATAAGTATTGACAACTTGTTAGTCCTT  
CCAACAAACCTGAACAAATTGATGAAGGACTTGCAATTGGCACATTGCTGCGATGCCCTTGGCAACTGCCACTCCT  
CATGGGCTAATCTGGAGTTCTACAGCGTCTGCCCTCTGCGACTTGGTTCTGTAGTCCTGCCCTTTCA  
GCTGGCTAGGGAGAATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTCAAAGACTTGTGATTACCTCAG  
AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGAGAACCAATGGAAAAATGATTGAAACTAAGACA  
AACAGAACTGAAACTGACTCGAAGGCAGCCTATGTGAGATACTTCAATAGCTGCCCTCTCTTCAGGGTTCTT  
GTGGTTTTATCTGCTCCCTATGCACTAAAGGAATCATCCTCCGGAAAATTACACCACATCTCATTCT  
GCATTGTTCTGCCATGGCGTACTCGCAATTCCCTGGCTGTACAAACATGGTATGACTCTTGGACAATAAA  
CAAACAGGATTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAAAGACTACAGAACTAGTGTGAG  
AATGTAACAGCCTCTGGAGGGATTGGGAATTATTGAGAAAGCAAACAAACAATAACAATAGAAAAACTT  
CTAATGGTATGACAGCCTCTTCACTTCTGGTACTCCTGTCCTGAAAGATATTCAAGAT  
AGAAAAGAGGACAGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGTATGATGATCATGGCGAG  
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCATCAGCTTGTGAGCCATTAGTTGATCATGCCCGTA  
CCATCAAGGAGAACATAATCTCGGCGTCACTTACGACCGACTCCGCTATCGCTGGTATTAGGCCTGTCAGTTGGA  
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTCACCTGATTGGCCTCGATACGCTAACGATCCACCGG  
TCAAAAGTTTCACATAATTCTTACCTCTTGAATTGATGACGCTCTGTATCTATATTGATCATTG  
GAAACACCAATGATATTCTTAATGGCCTGGCATATCTGGAAAATGATAACACAATGAAATTCTTCACTGT  
GCTTAATTACCCCTCTGAATTCTCCATTCTCCATAATCATCATTACAACGACTCTGGAAATAAACCATCATT  
ATTAACCTATTCAAATCACCGCT

FIG.42



153 bp PTM24 Binding Domain:

Nhe I                    153 bp BD underlined  
GCTAGC-AATAATGACGAAGCCGCCCTCACGGCTCAGGATTCACTTGCCCTAACATTATCATCCCTAAAGCAGAAAGTCATA  
  
TTCTTATTGTAAAGATTCTATTAAACTCATTTGATTCAAATATTAAATACTCCCTGTTCACCTACTCTGCTATGC

Sac II  
AC-GGGGG

FIG. 43A



Trans-splicing domain

AATAATGACGAAGCCGCCCTCACGCTCAGGATTCACTGCCCTCCAATTATCATCTAAGCAGAAGTGTATATTCTTA  
TTTGTAAAGATTCTATTAACTCAATTGATTCAAATATTAAAATACTTCCTGTTACCTACTCTGCTATGCACCCGC  
GGAACATTATTATAACCTGCTCGAATACTAACTGGTACCTCTTCTTTTGATATCCTGCAG

Exons 10-24

ACTTCACTCTAATGATTATGGAGAACTGGAGCCTTCAGGGTAAAATTAAAGCACAGTGAAGAATTCTATTCT  
GTTCTCAGTTTCCCTGGATTATGCCCTGGCACCATAAAGAAAATATCATCTTGGTTCCTATGATGAATATAGATA  
CAGAACGCTCATCAAAGCATGCCAAGTAGAAGAGGACATCTCAAGTTGAGAGAAAAGACAATATAGTTCTGGAGAA  
GGTGAATCACACTGAGTGGAGCTAACGAGCAAAATTCTTAGCAAGAGCACTATACAAAGATGCTGATTGATT  
TATTAGACTCTCCTTGGATACCTAGATGTTAACAGAAAAAGAAATTGAAAGCTGTCCTAAACTGATGCC  
TAACAAAATAGGATTTGGTCACTCTAAAATGAAACATTAAAGAAAAGCTGACAAAATATTAAATTGATGAAGGT  
AGCAGCTATTTATGGACATTTCAGAACTCCAAAATCTACAGCCAGACTTAGCTCAAAACTCATGGATGTGATT  
CTTCGACCAATTAGTCAGAAAGAAGAAATTCAATCCTAACTGAGACCTACCCGTTCTCATTAGAAGGAGATGC  
TCCTGTCCTGGACAGAAACAAAAACATCTTAAACAGACTGGAGAGTTGGGGAAAAAGGAAGAATTCTATT  
CTCAATCCAATCAACTCTACGAAAATTCCATTGCAAAAGACTCCCTACAAATGAATGGCATCGAACAGGATT  
CTGATGAGCCTTAGAGAGAAGGCTGCTTAGTACAGATTCTGAGCAGGGAGAGGCGATACTGCCCTCGCATCGCG  
GATCAGCACTGGCCCCACGCTTCAGGACGAAGGAGGAGCTGTCCTGAACCTGATGACACACTCAGTTAACCAAGGT  
CAGAACATTACCGAAAGACAACAGCATCCACACAAAAGTCACTGCCCTCAGGCAAACATTGACTGAACGGATA  
TATATTCAAGAAGGTTATCTAAGAAACTGGCTGGAAATAAGTGAAGAAATTACGAAGAACACTAAAGAGTGCTT  
TTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTCGATATAATTACTGTCACAAAGAGCTT  
ATTTTGCTAATTGCTGTTAGTAAATTCTGGCAGAGTGGCTCTTGGTTGCTGGCTCCTTGGAA  
ACACTCCTCTCAAGACAAAGGAATAGTACTCATACTAGAAATAACAGCTATGAGTATTACCCAGCACCAGTT  
GTATTATGTGTTTACATTACGTGGAGTAGCCGACACTTGTCTGCTATGGATTCTCAGAGGTCTACCACTGGT  
CATACTCTAATCACAGTGTGAAAATTACCCACAAATGTTACATTCTGTTCAAGCACCTATGTCACCCCTCA  
ACACGGTGAAGCAGCTGGATTCTTAATAGATTCTCAAAGATATGCAATTGGATGACCTCTGCCCTTACCAT  
ATTGACTTCATCCAGTTGTTATTAAATTGTGATTGGAGCTATGAGTTGCGAGTTTACAACCCCTACATCTTGT  
GCAACAGTCCAGTGAGTGGCTTTATTATGTTGAGAGCATTTCTCCAAACCTCACACCAACTCAAACAACTGG  
AATCTGAAGGAGGAGTCCAATTTCACTCATCTGTTACAAGCTAAAAGGACTATGGACACTTCGTCCTCGGAGC  
GCAGCCTACTTGAACACTCTGTTCCACAAAGCTCTGAATTACATACTGCCAAGTGGTTCTTGTACCTGTCACACTG  
CGCTGGTCCAATGAGAATAGAAATGATTTGTCATCTTCTCATGGTACCTCATTTCAATTAAACACAG  
GAGAAGGAGAAGGAAGAGTTGTTATTACCTGACTTTAGCCATGAATATCATGAGTACATTGCACTGGCTGAAACTC  
CAGCATAGATGTGGATAGCTGATGCCATCTGAGCCGAGCTTAAAGTATTGACATGCCAACAGAAGTAAACCT  
ACCAACTCAACCAACCATACAAGATGCCAAGTCTGAAAGTTGATTGAGAATTCACACGTGAAGAAAGATG  
ACATCTGCCCTCAGGGGCCAAATGACTGTCAAAGATCTCACAGCAAATACACAGAAGGTGGAAATGCCATATTAGA  
GAACATTCTCTCAATAAGTCTGGCCAGAGGGTGGCTTGGAAAGACTGGATCAGGAAAGAGTACTTGT  
TCAGCTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGATGATGGTGTCTGGATTCAATAACTTGCAC  
AGTGGAGGAAAGCCTTGGAGTGATACCACAGAAAGTATTATTTCTGGAACATTAGAAAAACTTGGATCCCTA  
TGAACAGTGGAGTGATCAAGAAATATGAAAGTGGAGATGAGCTGGCTCAGATCTGATAGAACAGTTCTGG  
AAGCTTGAATTGTCCTTGAGGGCTGTCCTAACCCATGCCACAAGCAGTTGATGTGCTGGCTAGATCTG  
TTCTCAGTAAGGCCAGATCTGCTGCTGATGAACCCAGTCTCATTGGATCCAGTAACATACCAATAATTAGAAG  
AACTCTAAACAAAGCATTGCTGATTGACAGTAATTCTGTGAACACAGGATAGAACCAATGCTGGAATGCCAACAA  
TTTTGGTCAAGAAGAGAACAAAGTGGCCAGTACGATTCCATCCAGAAACTGCTGAACGAGAGGAGCCTTCCGGC  
AAGCCATCAGCCCCCTCCGACAGGGTGAAGCTTTCCCCACCGGAACCTCAAGCAAGTGCAAGTCAAGCCCCAGATTG

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAACAGAGTGCAAGATACAAGGCTCATCATCATCATCATTAG

FIG. 43B